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ORIGINAL ARTICLES

Are there real benefits to implanting cardiac devices in patients with end-stage dilated dystrophinopathic cardiomyopathy? Review of literature and personal results

Alberto Palladino[1], Andrea A. Papa[2], Salvatore Morra[1], Vincenzo Russo[2], Manuela Ergoli[1], Anna Rago[2], Chiara Orsini[1], Gerardo Nigro[2] and Luisa Politano[1]

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Cardiomyopathy associated with dystrophinopathies – Duchenne muscular Dystrophy (DMD), Becker muscular dystrophy (BMD), X-linked dilated cardiomyopathy (XL-CM) and cardiomyopathy of Duchenne/Becker (DMD/BMD carriers – is an almost constant manifestation of these neuromuscular disorders and contribute significantly to their morbidity and mortality. Dystrophinopathic cardiomyopathy is the result of the dystrophin protein deficiency at the myocardium level, parallel to that occurring at the skeletal muscle level. Typically, cardiomyopathy begins as a "presymptomatic" stage in the first decade of life and evolves in a stepwise manner toward an end-stage dilated cardiomyopathy. Nearly complete replacement of the myocardium by fibrous and fatty connective tissue results in an irreversible cardiac failure, characterized by a further reduction of ejection fraction (EF < 30%) and frequent episodes of acute heart failure (HF). The picture of a severe dilated cardiomyopathy with intractable heart failure is typical of dystrophinopathies. Despite an appropriate pharmacological treatment, this condition is irreversible because of the extensive loss of myocites. Heart transplantation is the only curative therapy for patients with end-stage heart failure, who remain symptomatic despite an optimal medical therapy. However there is a reluctance to perform heart transplantation (HT) in these patients due to the scarcity of donors and the concerns that the accompanying myopathy will limit the benefits obtained through this therapeutic option. Therefore the only possibility to ameliorate clinical symptoms, prevent fatal arrhythmias and cardiac death in dystrophinopathic patients could be the implantation of intracardiac device (ICD) or resynchronizing devices with defibrillator (CRT-D). This overview reports the personal series of patients affected by DMD and BMD and DMD carriers who received ICD or CRT-D system, describe the clinical outcomes so far published and discuss pro and cons in the use of such devices.

Key words: dystrophinopathic cardiomyopathy, Duchenne muscular dystrophy, Becker muscular dystrophy, intracardiac devices, Duchenne/Becker carriers

Introduction

Dystrophinopathies are X-linked muscular dystrophies caused by mutations in the dystrophin gene, located at Xp21, that encodes for the sarcolemmal protein dystrophin virtually present in all tissues, but most abundant in skeletal muscle cells and heart (1, 2). Dystrophin provides the connection between the so called dystrophin-glycoprotein complex on the sarcolemma and the intracellular actin filaments, transmitting forces generated by the sarcomere contraction to the extracellular matrix (3, 4). Absence, reduced levels or abnormal structure of dystrophin lead to membrane fragility, making muscle fibres more prone to injury during contraction. As muscle disease progresses, muscle repair cannot adequately compensate for damage, leading to necrosis of skeletal and cardiac myocytes and the progressive replacement by fibrofatty tissue (5). Dystrophinopathic cardiomyopathy is the result of the dystrophin protein deficiency at the myocardium level, parallel to that occurring at the skeletal muscle level. Typically, cardiomyopathy begins as a "presymptomatic" stage in the first decade of life and evolves in a stepwise manner toward an end-stage dilated cardiomyopathy. Nearly complete replacement of the myocardium by fibrous and fatty connective tissue results in an irreversible cardiac failure, characterized by a further reduction of ejection fraction (EF < 30%) and frequent episodes of acute heart failure (HF) (6-11). Cardiac death usually occurs from systolic dysfunction, that represents the end stage of dystrophinopathic cardiomyopathy (DCM) or the onset of fatal arrhythmias.

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Dystrophinopathies can present with four clinical pictures, Duchenne muscular dystrophy (DMD), the more severe form, Becker muscular dystrophy (BMD), the more benign form, the X-linked dilated cardiomyopathy (XL-DCM) (2, 9) and the cardiomyopathy of DMD/BMD carriers (11). They are characterised by different pathogenic conditions that result in variable degrees of skeletal muscle and myocardial dysfunction. Having a better management of the ventilatory failure led to an increase in survival rates in these patients (12-14), heart failure remains an important contributor to the mortality. Despite the high incidence of end-stage DCM, there is a reluctance to perform heart transplantation (HT) in these patients due to the scarcity of donors and the concerns that the accompanying myopathy will limit the benefits obtained through this therapeutic option (15, 16) .

In patients with New York Heart Association (NYHA) class III, ambulatory class IV systolic heart failure (HF) and recently class I and II, with electrocardiographic evidence of ventricular dyssynchrony, cardiac resynchronization therapy with defibrillator (CRT-D) has been shown to a) improve quality of life and functional status, b) reduce heart failure-related hospitalizations, and c) prolong survival (17-26). Implantable cardioverter defibrillators (ICDs) have revolutionized the primary and secondary prevention of patients with heart failure (27-30) and ventricular arrhythmias (31-33). The implantation of an ICD is considered in cases of non-sustained ventricular tachycardia unresponsive to drug treatment (usually beta-blockers) while a CRT-D system is preferred in presence of a drug-resistant heart failure associated to a left branch bundle block (LBBB), especially when conventional measures are ineffective (27). Biventricular pacing is able to synchronize left ventricular contractions, improve left ventricular function, and decrease left ventricular filling pressure. CRT-D is an adjuvant treatment for patients with post-ischemic dilated cardiomyopathy and symptomatic, drug refractory heart failure, providing both acute and long term hemodynamic and functional improvement (27, 31-33). Recent studies have reported in these patients an improvement of symptoms accompanied by the reduction of left ventricular volumes mitral regurgitation, a marker of the ventricle remodelling and the increase of LV ejection fraction (LVEF).

As tachy-arrhythmias and mechanical dyssynchrony are frequent in dystrophinopathic patients with end stage dystrophin-associated myocardial dysfunction (34-43), the implantation of ICD or CRT-D could be indicated to ameliorate clinical symptoms and prevent life-threatening arrhythmias and cardiac sudden death also in these patients.

However, few data are available about cardiac device implantation in dystrophinopathic patients. Takano et al. 44), Fassoyl et al. (45) and Kuru S et al. (46) reported on isolate cases of DMD patients receiving a pacemaker implant for complete atrioventricular block or sinus node dysfunction in 1997, in 2005 and in 2012, respectively. Stollberger et al. (47) reported a case of a 40-year-old BMD patient with severe heart failure (LVEF 25%) who benefited from CRT-D. However no amelioration was found regarding the LVEF three months after the CRT therapy and the patient died 16 weeks after implantation. Andrikopoulos et al. (48) reported the case of a BMD patient with advanced heart failure due to non-ischemic cardiomyopathy (NICM), with noncompaction morphology of the left ventricle, and associated electrical and mechanical dyssynchrony, who became nearly asymptomatic (NYHA class I) shortly after implantation, with an improvement in LV function documented by 3D-echocardiography. CRT-D has been successfully experienced in a 34 year old DMD, presenting with asthenia, leg oedema and ascites, moderate left ventricle dilation, decreased ejection fraction (30%) and a significant arterial pulmonary pressure (57 mmHg). One year before the patient was implanted of dual chamber pacemaker because of a complete atrio-ventricular block. Upgrade from a dual-chamber to a biventricular pacemaker produced, one month after, stabilization of systolic function, regression of interventricular and intra-ventricular asynchrony and decrease of pulmonary artery pressure (40 mmHg). After 5 years of follow-up, the ejection fraction improved to 45% (49).

However – except for these isolated case reports - no definitive figures exist in literature concerning the number of patients with dystrophinopathic cardiomyopathy who received ICD or CRT-D and their outcome, nor clear indications in the current guidelines that consider the use of cardiac devices as an option for dystrophinopathic patients with end-stage dilated cardiomyopathy.

Aim of this overview is to a) report the personal series of patients affected by DMD and BMD and DMD carriers who received ICD or CRT-D, b) describe the clinical outcomes so far published and c) discuss pro and cons in the use of such devices in this selected population.

Patients and methods

Patients

We retrospectively analyzed data from 18 dystrophinopathic patients followed at the Cardiomyology and Medical Genetics of the Luigi Vanvitelli Campania University, 5 affected by DMD, 10 by BMD and 3 DMD carriers, who were implanted – after informed consent - with ICDs or an CRT-Ds in the period June 2007-November 2018. The study was approved by the local ethical Committee.

Since diagnosis, based on clinical and genetic analysis, all patients undergone periodical evaluations that included cardiologic assessment, standard and dynamic

ecg, echo-color-doppler-cardiogram and electrophysiological study (SEF) when necessary. The evaluations were performed every 3-months, according to the clinical presentation. All patients were on cardiological treatment, in particular ACE-inhibitors, beta-blockers, antiarrhythmic drugs and anticoagulants.

Methods

ECG. Standard 12 lead ECG was obtained in all patients; QRS duration was measured manually. The presence of fibrosis, arrhythmias or bundle branch blocks was also noted.

24-hour Holter monitoring ECG. Hear rate (HR) and presence and type of arrhythmias were assessed by 24-hour Holter monitoring system.

Echocardiogram. Left ventricular volumes, mass and global function were assessed via standard planimetry techniques using semi automated computer software (Philips SONOS 5500 Imaging System, Netherlands) by expert readers (AP, SM). Ventricular volumes, mass, ejection fraction as far as ratio EDV/m^2 were tabulated for each subject.

The indication for a device implantation was made in presence of subjective symptoms (dyspnoea, fatigue, reduced exercise tolerance) corresponding to a III-IV NYHA class, EF ≤ 35% and/or cardiac dilation (ratio EDV / m^2 > 70) or in presence of arrhythmias. The implant of devices was performed under local anesthesia obtained by subcutaneous administration of lidocaine.

Results

The results are shown in Tables 1 and 2.

Table 1 shows the cardiological features of patients enrolled in the study, collected at the last visit before implantation. All DMD patients and 50% of BMD patients were chair-bound. The mean age of loss of ambulation (LoA) was 13.3 ± 1.6 years for Duchenne and 42.7 ± 11.2 years for Becker patients.

Not sustained ventricular tachycardia (NSVT) was reported in 7/18 (38.8%) and ventricular ectopic beats (VEB) in 4/18 (22.2%) patients considered as a whole. Atrio-ventricular blocks were observed in 3/18 patients (16.7%). Postero-lateral fibrosis was observed in all Duchenne patients and only in one Becker (patient n. 8), at the posterior level. A left bundle branch block (LBBB) was present in 6/18 patients (33.3%), 1 with Duchenne, 3 with Becker and 2 carriers. Before implantation, the mean

Table 1. Cardiological parameters of patients before implantation.

Patient number	LoA in years	Age at the device implantation	Ejection fraction in % (n.v. > 55)	EDV/m^2 (n.v. < 70)	Presence/type of arrhythmias or BBB and fibrosis	Type of device implanted
DMD n. 1	15y 10m	15y 10m	35	166	NSVT; postero-lateral fibrosis	ICD
DMD n. 2	13y	23y 6m	30	108	NSVT; postero-lateral fibrosis	ICD
DMD n. 3	13y 8m	28y 11m	33	78	None; postero-lateral fibrosis	ICD
DMD n. 4	11y 5m	15y 7m	40	91	AVB 2:1; LBBB; postero-lateral fibrosis	CRT-D
DMD n. 5	12y 6m	26y 5m	35	108	NSVT; postero-lateral fibrosis	ICD
BMD n. 1		39y 8m	32	109	VEB; LBBB	CRT-D
BMD n. 2		51y 7m	28	127	None	ICD
BMD n. 3	52y	51y 7m	30	82	AVB 3rd degree	CRT-D
BMD n. 4		56y 4m	33	91	NSVT	ICD
BMD n. 5	40y	45y	40	155	NSVT	ICD
BMD n. 6	45y 2m	51y 7m	28	127	NSVT	ICD
BMD n. 7	51y 8m	51y 3m	33	111	AVB 1st degree; AVB 2nd degree, type 1 and type 2; RBBB	PM upgraded to ICD
BMD n. 8	24y 10m	33y 2m	35	118	VEB; posterior fibrosis	ICD
BMD n. 9		58y 4m	38	139	VEB	ICD
BMD n. 10		60y 10m	35	124	NSVT, LBBB	CRT-D
DMDc n. 1		54y 6m	31	147	LBBB	CRT-D
DMDc n. 2		55y 4m	37	147	VEB	ICD
DMDc n. 3		50y 8m	30	147	LBBB	CRT-D

DMD: Duchenne Muscular Dystrophy; BMD: Becker Muscular Dystrophy; DMDc: Duchenne Muscular Dystrophy carrier; LoA: loss of ambulation; EDV: end-diastolic volume; m^2= height in meters, elevated to the square; NSVT: Not sustained Ventricular Tachicardia; AVB: atrio-ventricular block; LBBB: Left Bundle Branch Block; RBBB: Right Bundle Branch Block; VEB: Ventricular ectopic beats.

Table 2. Comparison of cardiological parameters before and after implantation.

Patient number	Ejection fraction in % before implantation (n.v. > 55)	Ejection fraction in % post-implantation (n.v. > 55)	EDV/m² before implantation (n.v. < 70)	EDV/m² post implantation (n.v. < 70)	FU in months since the implantation
DMD n.1	35	32	166	166	5
DMD n.2	30	37	108	90	40
DMD n. 3	33	38	78	99	25
DMD n. 4	40	36	91	95	5
DMD n. 5	35	25	108	97	21
Mean ± SD	34.6 ± 3.6	33.6 ± 5.4	110.2 ± 33.6	109.4 ± 31.8	19.2 ± 14.8
BMD n.1	32	20	109	153	69
BMD n.2	28	35	127	153	66
BMD n. 3	30	35	82	85	3
BMD n. 4	33	33	91	91	5
BMD n. 5	40	21	155	169	53
BMD n. 6	28	31	127	145	76
BMD n. 7	33	31	111	95	136
BMD n. 8	35	28	118	138	67
BMD n. 9	38	30	139	127	40
BMD n. 10	35	35	124	99	42
Mean ± SD	33.2 ± 3.9	29.9 ± 5.5	118.3 ± 21.5	125.5 ± 30.6	55.7 ± 38.1
DMDc n. 1	31	28	147	236	71
DMDc n. 2	37	25	147	137	41
DMDc n. 3	30	25	147	172	96
Mean ± SD	33.7 ± 3.8	26.0 ± 1.7	147.0 ± 0	181.6 ± 50.2	69.3 ± 27.5

DMD: Duchenne Muscular Dystrophy; BMD: Becker Muscular Dystrophy; DMDc: Duchenne Muscular Dystrophy carrier; LoA: loss of ambulation; EDV: end-diastolic volume; m^2: height in meters, elevated to the square.

value of ejection fraction was 34.6 ± 3.6% in Duchenne, 33.2 ± 3.9% in Becker and 32.7 ± 3.8% in DMD carriers. The mean value of VTD/m2, a parameter considered as a marker of cardiac dilation, was 110.0 ± 33.6 in Duchenne, 118.3 ± 21.5 in Becker and 147.4 ± 0.05 in DMD carriers.

Twelve out of 18 patients received an ICD as cardiac device, while 1 Duchenne patient (n. 4), 3 Becker patients (n.1, n. 4 and n. 9) and 2 carriers (n. 1 and n. 3) received a CRT-D because the contemporary presence of mechanical dyssynchrony.

The device implantation was performed at a mean age of 21.8 ± 5.9 years in Duchenne patients, 50.3 ± 8.7 years in Becker patients and 53.5 ± 2.5 years in DMD carriers. The average duration in months of the follow-up was 19.2 ± 14.8 (range 5-40 months) for Duchenne patients, 55.7 ± 38.1 (range 3-136 months) for Becker patients and 69.3 ± 27.5 (range 41-96 months) for the DMD carriers.

Table 2 shows a comparison between data obtained before and after the implantation. The ejection fraction varied on average from 34.6 ± 3.6% to 33.6 ± 5.3% in Duchenne patients, from 33.2 ± 3.9% to 29.9 ± 5.5% in Becker patients and from 32.7 ± 3.8% to 26 ± 1.7% in DMD carriers. None of the three groups recovered normal values, rather we saw a stabilization of the starting values or more often a clear deterioration, particularly in Becker patients and DMD carriers. Similarly, the mean values of VTD/m^2 changed from 110 ± 33.6 to 109.4 ± 31,8 in Duchenne patients, from 118.3 ± 21.5 to 125.5 ± 30.6 in Becker and from 147.4 ± 0.05 to 181.0 ± 50.2 in DMD carriers, values clearly indicating a progression in the heart dilation in the last two groups.

A restrictive respiratory failure was present in all DMD patients with percentage of Forced Vital Capacity (FVC) ranging from 6 to 71% compared with the expected values. Only one Becker patient had a FVC equal to 60%, while the remaining had values ranging from 71 to 100%. Two out of DMD carriers had FVC values at about 60% of the expected ones.

During the follow-up 6/18 patients (33.3%) died. Three Duchenne and one Becker patients from respiratory failure, two carriers from intractable heart failure. The death occurred on average 22 months in DMD, 50 months in BMD and 60 months after implantation, respectively.

Despite these not encouraging results, 25% of patients referred they have got something positive out of

this situation in terms of cardiac symptoms and daily life activities.

Implant-related complications

Usually two types of major implant-related complications can occur: (1) In-hospital complications and (2) complications within 90 days of discharge. In-hospital complications include: in-hospital death; re-operation including generator, lead or pocket re-operation with incision and drainage of hematoma, seroma, or abscess; post-procedural shock; pericardial or pleural drainage; and infective endocarditis.

Post-discharge complications include: death within 30 days of discharge; re-operation for reasons reported above; re-hospitalization within 90 days with a primary diagnosis consistent with a device-related complication; infection (device infection, endocarditis, systemic infection); pneumothorax or pericardial effusion; pocket-related complications such as hematoma or wound dehiscence; venous obstruction or thrombo-embolism and other admissions for potentially serious device-related complications.

The occurrence of in-hospital and post-discharge complications have been estimated in about 8-8.5% of patients, with a slight prevalence for women (50), prevalently consisting in pleural and pericardial drainage and infections (50-52).

In our cohort of patients, only 1 BMD patient had implant-related complications consisting in a healing defect of the ICD pocket.

Discussion

Cardiac dysfunction in patients with Duchenne/Becker muscular dystrophy (DMD/BMD) and in DMD/BMD carriers is a leading cause of death, together with the onset of life-threatening arrhythmias. Implantable cardiac defibrillators and cardiac resynchronization therapy with defibrillator have been shown to dramatically decrease mortality in eligible adult population with congestive heart failure. Current therapeutic options for dystrophinopathic patients presenting heart failure are limited and no established standard of care for medical or device interventions are still available. Furthermore few studies sought to determine the feasibility of ICDs or CRT-Ds in DMD/BMD population, most of whom have normal QRS complexes. The data here reported, while seem to confirm the limited benefits from the use of this therapeutic approach, on the other hand show that 25% of patients have had a subjective improvement in their daily activities. The normality of QRS complex as well as the extensive postero-lateral fibrosis associated to dystrophinopathic cardiomyopathy are likely the cause of poor response to the treatment, at least in Duchenne patients. This suggests that it would be advisable - in determining the indications for implantation of the ICD and CRT-D for primary prevention of sudden cardiac death in Duchenne patients - to take into account not only the value of left ventricle ejection fraction, but also the features of the fibrosis of the left ventricle.

Patients with severe dystrophinopathy may be at risk for respiratory insufficiency because of diaphragm involvement and chest deformities; moreover, a device implantation may be problematic in these patients because of possible and serious mechanical and infective complications. Fayssoil et al. have recently (53) reported retrospective data on the risks related to ICD in muscular dystrophy patients ventilated by tracheostomy. They found 12 device implantations performed in 9 patients (5 DMD, 1 BMD and 3 DM1), at a mean age of 39.9 years ± 13.0. All patients were wheel-chair bound and tracheotomised. Concerning the type of the device, 6 were pacemakers (PM) and 6 CRT devices, including 2 CRT-D. They observed a high prevalence of early complications (16.6% pneumothorax) and an acceptable long-term infectious risk (8.3%).

A further major risk in these patients is general anesthesia (54), so that the most part of these operations are made under local anesthesia. In cases of trans-muscle access, Froyshteter et al. have recently suggested the use of unilateral pectoralis and intercostal nerve blocks, supplemented with intravenous sedation (55).

Because data about the pros and cos in using ICD and CRT-D in dystrophinopathic patients remain controversial, specific guidelines on device therapy, similar to those established for patients with acute and chronic heart failure by the European Society of Cardiology (ESC), the Heart Failure Association (HFA) of the ESC and the European Society of Intensive Care Medicine (ESICM) (56,57) are strongly advocated to expand and support the CRT indication in dystrophinopathic patients.

Conflict of interest

The Authors declare to have no conflict of interest.

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Novel *TRIM32* mutation in sarcotubular myopathy

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Tripartite motif-containing protein 32 (*TRIM32*) is a member of the TRIM ubiquitin E3 ligases which ubiquitinates different substrates in muscle including sarcomeric proteins. Mutations in *TRIM32* are associated with Limb-Girdle Muscular Dystrophy 2H. In a 66 old woman with disto-proximal myopathy, we identified a novel homozygous mutation of *TRIM32* gene c.1781G > A (p. Ser594Asn) localised in the c-terminus NHL domain. Mutations of this domain have been also associated to Sarcotubular Myopathy (STM), a form of distal myopathy with peculiar features in muscle biopsy, now considered in the spectrum of LGMD2H. Muscle biopsy revealed severe abnormalities of the myofibrillar network with core like areas, lobulated fibres, whorled fibres and multiple vacuoles. Desmin and Myotilin stainings also pointed to accumulation as in Myofibrillar Myopathy. This report further confirms that STM and LGMD2H represent the same disorder and suggests to consider *TRIM32* mutations in the genetic diagnosis of Sarcotubular Myopathy and Myofibrillar Myopathy.

Key words: *TRIM32*, LGMD2H, sarcotubular myopathy, spheroids bodies, myotilin, desmin

Introduction

The *TRIM32* gene is composed of two exons encoding for a protein of 653 amino acids which is a member of the TRIM ubiquitin E3 ligases. *TRIM32* is characterized by a N-terminal conserved motif composed of a RING domain followed by a B-box and a Coiled-Coil domain, while its C-terminal portion presents 6 NHL repeats (1). The RING domain confers E3 ligase activity to *TRIM32*, the B-box and Coiled-Coil domains help the correct folding of the protein and the C-terminal domain mediates the interaction of *TRIM32* with its substrates. The main role of *TRIM32* consists in ubiquitination of different specific substrates (2, 3). Among these are included many muscular proteins such as actin, alpha-actinin, desmin, tropomyosin and dysbindin, thus indicating a role of *TRIM32* in promoting ubiquitin-dependent degradation of target proteins. Interestingly, *TRIM32* was reported to localize around the Z-line in skeletal muscle of guinea pig, showing a potential role of *TRIM32* in the maintenance and physiology of the sarcomere (4). Nevertheless *TRIM32* is involved in ubiquitination of cell cycle regulators (c-Myc, MYCN, p53) and the cell growth and transformation factor, Abi2 (5, 6), involving *TRIM32* in other signaling mechanisms such as the regulation of muscle satellite cells renewal and differentiation.

TRIM32 mutations were initially described in the Manitoba Hutterite population (41 patients) of North America presenting with a LGMD2H phenotype and the first mutation identified was the c.1459G > A (p. Asp487Asn) (7). LGMD2H is an autosomic recessive limb girdle muscular dystrophy associated with mildly to moderately increased creatine kinase (CK), presenting with a wide clinical presentation spectrum, ranging from virtually asymptomatic patients to rarely wheelchair-bound in the late course of their disease. The same mutation reported by Frosk et al. (7) was also identified in four pa-

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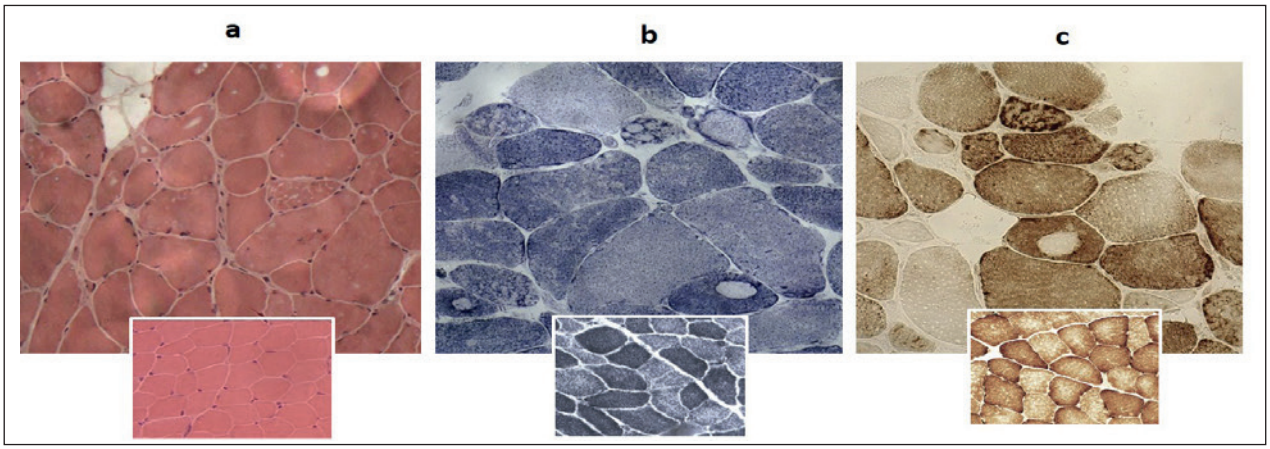

Figure 1. Muscle biopsy. a-c) Representative images of H&E (a), NADH (b) and COX (c) stainings from patient muscle biopsy are shown. Lobulated fibres, whorled fibres and multiple vacuoles containing amorphous material are evident. In small boxes pictures from a normal control biopsy with the same staining are presented for comparison.

tients, affected by Sarcotubular Myopathy (STM), a form of autosomal recessive myopathy (8). Schoser et al. (8) hypothesized that STM and LGMD2H represent different severity presentations of the same disease, since STM and LGMD2H present with clinical and histological overlapping findings. Later, additional mutations in *TRIM32* has been identified in LGMD2H patients of non-Hutterite origins (9-14).

Patients harbouring mutations in *TRIM32* share common features at muscle biopsy, such as increased fiber size variation, marked increase of internal nuclei and typical small, irregularly slit-shaped vacuoles that appeared empty. Electron microscopy showed the vacuoles to originate from focal dilations of the sarcoplasmic reticulum. The membranes limiting the vacuoles also showed sarcoplasmic reticulum-associated ATPase reactivity, confirming that the vacuoles arose from the cytoplasmatic organelles.

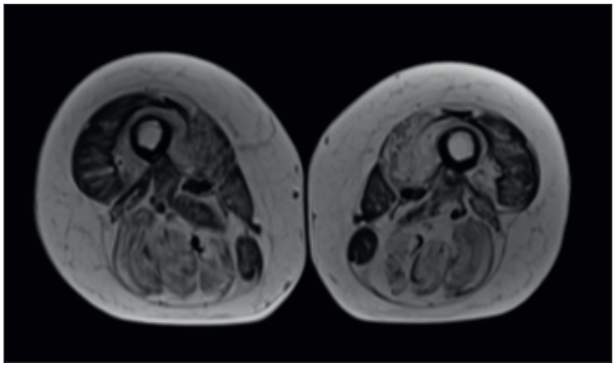

Figure 2. Muscle MRI. Muscle MRI of lower limbs showed a severe involvement of adductors longus, magno and brevis (Score 4), glutei and tight posterior muscles (Score 3).

In some muscle fibres small vacuoles were tightly packed and the membranes were partially disrupted resulting in larger vacuoles. Based on these findings muscle biopsies from patients with mutations in *TRIM32* gene have been defined as Sarcotubular Myopathy pattern (8, 15-18). Occasionally mild increase of endomysial fibrous connective tissue, necrotic fibers and fiber splitting were also reported. In two further reports, authors defined unspecific findings at muscle biopsy with no signs of sarcotubular aggregates in patients with TRIM2 gene mutations (12, 14).

The present work describes the clinical, histological and radiological features of a LGMD2H patient due to a novel homozygous mutation in the *TRIM32* gene with a typical Sarcotubular Myopathy pattern at muscle biopsy.

Case report

The proposita, a 66-year- old woman, was the second child of healthy unrelated parents. She was born at term after uneventful pregnancy and normal delivery. Psychomotor development was reported normal and she did not refer motor defects during her childhood nor early adulthood.

At 40 years she incidentally documented a moderate hyperckemia (4X) without any muscle symptoms. When she was 46 years old she showed proximal weakness, particularly at the pelvic girdle, leading to weakness while climbing stairs. In the following 20 years she presented with a slowly progressive lower limb girdle muscle weakness, being the upper limb performances less affected. She has never complained about respiratory symptoms nor cardiological involvement occurred.

At 49 years, a muscle biopsy from quadricep was performed, which revealed severe abnormalities of the

myofibrillar network with core-like areas, lobulated fibres, whorled fibres and multiple vacuoles containing amorphous material (Fig. 1a-c). The histological findings pointed out a sarcotubular myofibrillar disorder.

Latest neurological examination at the age 66 showed minimal hypotrophy at scapular girdle muscles without muscle strength impairment, pelvic girdle muscle weakness (quadriceps MRC 4, adductors MRC 3), waddling gait aided with a stick. Deep tendon reflexes were reduced in all limbs. Pseudo-hypertrophy of calves was evident. Rigid spine was also noted. Respiratory muscles function was spared with normal spirometry. Lower limb muscle MRI was performed at 64 years according to the protocol previously described (19). The MRI disclosed complete atrophy and fat substitution of adductors longus, magnus and brevis (Goutallier score 4), severe involvement of glutei and hamstrings muscles (Goutallier score 3) and a selective sparing of gracilis, sartorius and quadriceps muscles (Goutallier Score 2) (Fig. 2).

Recently this case was included in a group of undiagnosed muscular dystrophy patients to be analyzed by Limb Girdle Panel, an extended NGS testing panel which investigates the coding regions of 44 genes linked to LGMDs. We identified a novel homozygous mutation of *TRIM32*, NM_012210.3: c.1781G > A, (p. Ser594Asn) c.1781G > A/p.Ser594Asn, localized in the C-terminus NHL domain. Unfortunately patient's parents were not available for segregation study. Thus, to exclude a possible deletion of the second allele as previously reported (17) we performed qualitative and quantitative analysis of *TRIM32* cDNA and we didn't identify alternative transcripts. (data not shown).

The molecular model of *TRIM32* refined with YASARA (Yet Another Scientific Artificial Reality Application; www.yasara.org) showed that this mutation alters specifically the correct conformation of the NHL domain (Fig. 3a). Mutations of this domain have been also associated to Sarcotubular Myopathy (STM), a form of distal myopathy with peculiar features in muscle biopsy, now considered in the spectrum of LGMD2H.

Since different muscular-relevant proteins have been identified as *TRIM32* substrates, Desmin and Myotilin stainings were performed and the results pointed to accumulation of these proteins within the muscle fibers (Fig. 3b-c). Furthermore, Western blot analysis with anti-*TRIM32* antibody showed a modest reduction of *TRIM32* expression compared to the control (Fig. 3d).

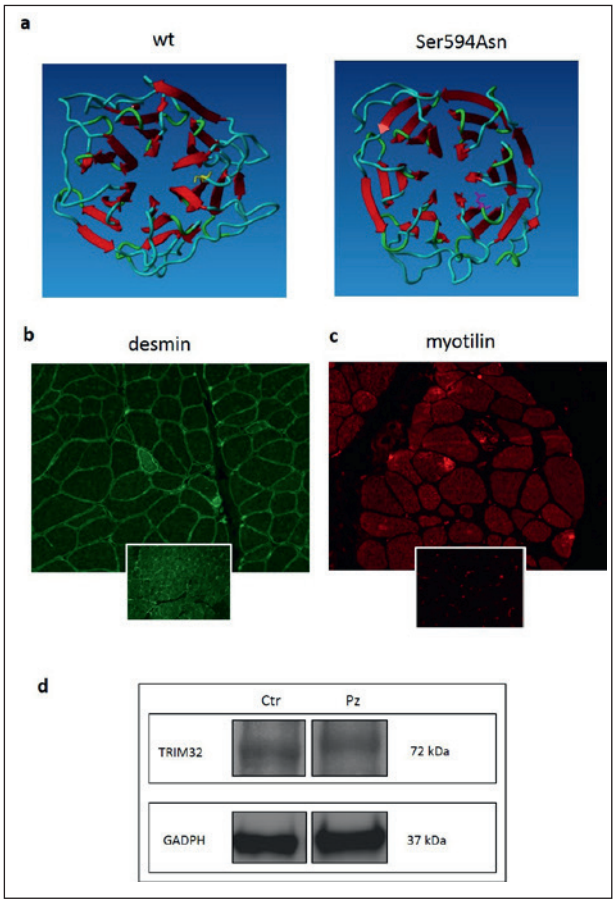

Figure 3. *TRIM32* and its substrates. a) *TRIM32* modeling of WT and mutated protein was performed YASARA. Mutation p.Ser594Asn alters specifically the correct conformation of the NHL domain. b-c) Desmin and Myotilin stainings pointed out accumulation of these proteins within the muscle fibers. In small boxes pictures from a normal control biopsy immunofluorescence with same antibody d) *TRIM32* protein levels were analyzed by Western blot. In the patient, the amount of *TRIM32* protein in muscle was only slightly reduced compared to control

Discussion

We describe a novel mutation in *TRIM32* gene in an adult patient who presented with a mild limb girdle muscle weakness without respiratory nor cardiac involvement. Muscle biopsy was suggestive of sarcotubular myopathy or myofibrillar myopathy. MRI findings are similar to those described in literature (12, 14), showing a preferential affection of the posterior thigh compartment with the sparing of sartorius, gracilis and the adductor longus.

Recently Johnson et al. (14) described 9 patients carrying pathogenetic mutations in *TRIM32*. Muscle biopsies showed non-specific myopathic or dystrophic changes in most patients, whereas scattered vacuoles were noted only in 3 cases. These were described as rimmed vacuoles containing basophilic membranes. Our patient, dissimilarly from this report, displays mainly myofibrillar network abnormalities with core-like areas, lobulated fibers, whorled fibers. Additionally, multiple

large vacuoles containing amorphous material/deposits similar to cytoplasmic hyaline bodies or spheroid bodies are present.

Interesting, spheroids bodies have been described in association with myotilin mutations (20) and a myotilin defect is also responsible of a form of Myofibrillar Myopathy. Hyaline bodies myopathies is a blurred definition of pathology alterations that, over the years, has been linked to mutations in several genes such as MYH7 in the form of myosin storage myopathy and FHL1 in the form of reducing bodies. In the whole, these myopathies with spheroid bodies, hyaline bodies but also cap and cytoplasmic bodies, have been referred as Surplus Protein Myopathies indicating an excess of proteins present in a granular or filamentous form (21). In this scenario we speculate that the mutation here described abolishes the interaction between *TRIM32* and its target proteins, which leads to a decreased ubiquitination and degradation by the proteasome machinery, thus inducing their accumulation to greater concentrations in the cytoplasm. To our knowledge, this is the first report which identified some of the proteins accumulated in the vacuoles in patient with *TRIM32* mutation. Indeed, immunostainings for Desmin and Myotilin, which are substrates of the *TRIM32* E3 ligase, pointed to their accumulation in the cytoplasm. We interpreted these findings as result of altered ubiquitination of these proteins which are known substrates of *TRIM32*.

Furthermore our patient, harbouring a mutation localized in the NHL domain, strengthens the previous findings according to which all the point mutations associated with LGMD2H are clustered in the C-terminus NHL domain, thus indicating a possible specific activity/property intrinsic to the NHL domain in the muscular tissue. The NHL domain is postulated to be critical for the recognition of protein targets to be ubiquitinated by this E3 ligase.

In conclusion, this report further confirms that STM and LGMD2H represent the same disorder and suggests to consider *TRIM32* mutations in the genetic diagnosis of Sarcotubular Myopathies and Myofibrillar Protein Myopathies. We also provided evidence that Desmin and Myotilin represent the contents of the vacuoles in a muscle biopsy from a *TRIM32* mutated patient.

Conflict of interest

The Authors declare to have no conflict of interest.

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An unusual presentation of scleromyxedema as inflammatory myopathy

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Scleromyxedema is a rare cutaneous mucinosis with frequent extracutaneous manifestations. Myopathy in scleromyxedema is a poorly recognized syndrome among neurologists and can mimic idiopathic and connective tissue disease-associated inflammatory myopathy. Diagnosis is suspected by the characterization of the skin lesions and clinched by skin and muscle biopsies. Here, we report a patient with scleromyxedema and myopathy with the characteristic histopathological feature of mucin deposition in skin biopsy. Her muscle biopsy showed a picture consistent with scleromyxedema myopathy with vacuolar and inflammatory changes. The association with paraproteinemia, propensity to life-threatening central nervous system disease and good response to intravenous immunoglobulin necessitate the accurate diagnosis of this condition.

Key words: scleromyxedema, monoclonal gammopathy, inflammatory myopathy, vacuolar myopathy, scleroderma

Abbreviations

IVIg: Intravenous immunoglobulin
MRC: Medical Research Council

Introduction

Scleromyxedema is a rare cutaneous mucinosis characterized by dermal mucin deposition and fibroblast proliferation. This disease commonly affects middle-aged people and shows no sex predilection (1). An increased production of mucin and hyaluronic acid in scleromyxedema is presumed to result from cytokine-mediated fibroblast stimulation, possibly from an abnormal plasma cell clone (2). The diagnosis is established by satisfying the criteria of (i) generalized papular and sclerodermoid eruption, (ii) mucin deposition, fibroblast proliferation, and fibrosis in skin histopathology, (iii) monoclonal gammopathy and (iv) absence of thyroid dysfunction (3).

Extracutaneous manifestations including nervous system involvement are frequent and potentially life-threatening. Neurological syndrome manifests as encephalopathy, neuropathies, stroke, seizures, acute psychosis or rarely coma ('dermato-neuro syndrome') (1). Myopathy and dysphagia are the other common presentations which are apparent in up to 50% patients (4, 5). These symptoms in combination with cutaneous lesions raise the alternate diagnostic possibilities of systemic sclerosis-associated myositis, dermatomyositis, and myxedema. The diagnosis of scleromyxedema can be missed in this setting owing to the rarity of the disease. Herein, we discuss the case of a lady who presented with dysphagia and myopathy with the typical skin lesions of scleromyxedema.

Case report

A 38-year-old woman presented with a two-year history of progressive symmetric proximal lower and upper limb weakness. She gradually developed dysphagia to solids, hypophonic nasal speech and neck weakness with severe weight loss. One year prior to the onset of the weakness, she had noted painless nodular skin lesions on her fingers, face and trunk with diffuse skin thickening for which she was seen by a dermatologist. She underwent skin biopsy and was prescribed topical treatment,

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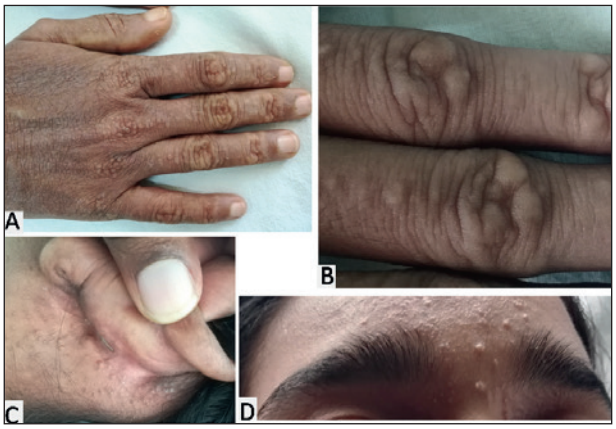

Figure 1. Indurated and hyperpigmented skin of dorsum of hand with multiple non-erythematous, closely-placed, dome-shaped, firm, papular and nodular lesions with a waxy appearance (A). The characteristic "doughnut sign" (B) with an elevated rim of thickened skin and central depression over the interphalangeal joints. Papular lesions involving the post-auricular region (C) and forehead (D).

but was then lost to follow up. At presentation to our center, she was ambulant but needed considerable help for rising and climbing.

On examination, she was emaciated and had induration of the skin of her hands, forearms, neck, upper trunk, and thighs. She had multiple non-erythematous, closely-placed, dome-shaped, papular and nodular waxy lesions over the dorsum of the hands, post-auricular region and between the eyebrows (Fig. 1). The mobility of the fingers was restricted suggesting sclerodactyly. There were no telangiectasias or calcinosis. Neurological examination showed symmetric palatal and tongue weakness with tongue atrophy. Symmetric weakness of neck flexors (Medical Research Council (MRC) scale 2/5), triceps (2/5), biceps (4/5), hip flexors (4/5) and quadriceps (3/5) was noted. Deep tendon reflexes and sensory examination were normal.

Serum creatinine phosphokinase was elevated (685 U/L, normal 26-192 U/L) and thyroid function tests were normal. Electromyography showed myopathic potentials with fibrillations and positive sharp waves. Peripheral nerve conduction study showed symmetrically reduced peroneal nerve compound muscle action potentials recording from extensor digitorum brevis muscles, with normal pickup from tibialis anterior muscles and inelicitable F waves from peroneal nerves. Rest of the motor and sensory conduction parameters were normal. Serum antinuclear antigen and extractable nuclear antigens were negative. Immunofixation electrophoresis showed monoclonal bands in IgG and lambda regions. Bone marrow biopsy ruled out plasma cell proliferation.

Skin biopsy from left forearm was reviewed which showed fibrosis of dermis with thick collagen bundles, loss and fragmentation of elastic fibers, and colloidal iron-positive acid mucin deposition (Fig. 2A-C). Muscle biopsy from left quadriceps showed loss of fascicular architecture with endomysial fibrosis and adipose tissue infiltration. Many myofibers exhibited large cytoplasmic vacuoles that failed to stain with periodic acid-Schiff, Oil red O and mucin stains (colloidal iron, Alcian blue and toluidine blue). However, acid mucin deposition was noted in the endomysial and perimysial connective tissue along with chronic inflammatory cell infiltrate (Fig. 2D-I). These histopathological features were consistent with vacuolar and inflammatory myopathy associated with scleromyxedema.

She was initiated on monthly intravenous immunoglobulin (IVIg) at 2 g/kg and oral prednisolone (1 mg/kg). After 3 months of therapy, she had nearly 50% improvement of muscle power with proximal limb power improving to MRC grade 4+ and neck flexion improving to grade 3. She had subjective improvement in swallowing, but no objective change was noted in the swallow assessment or palatal and tongue excursion. Skin lesions over the face and hands improved but did not completely disappear. She is currently maintained on oral thalidomide and prednisolone.

Discussion

We have described a rare case of scleromyxedema-associated myopathy which can present as a close differential diagnosis of idiopathic inflammatory myopathy. When the dominant presentation is extracutaneous, as in our patient, diagnostic labelling can be tenuous.

The characteristic skin lesions of scleromyxedema are non-pruritic, flat-topped, waxy, firm papules affecting the distal forearms, neck, and face, sparing the palm and mucous membranes. Typically, the skin is indurated with reduced mobility of jaw and extremities (6). The cutaneous lesions mimic localized scleroderma, systemic sclerosis, scleredema, nephrogenic systemic fibrosis, and lichen myxoedematosus. The diagnosis is clinched by skin biopsy which characteristically demonstrates dermal mucin accumulation, increased collagen deposition, and fibroblast proliferation (7).

The diagnosis becomes challenging when the dominant presentation is proximal limb-girdle and bulbar weakness as in our patient. In the absence of skin lesions, the diagnostic considerations would include myopathies such as oculopharyngeal myopathy, myotonic dystrophy and inflammatory myopathy, neuromuscular junction disorders and anterior horn cell disease (8). Skin lesions would prompt the consideration of systemic sclerosis as-

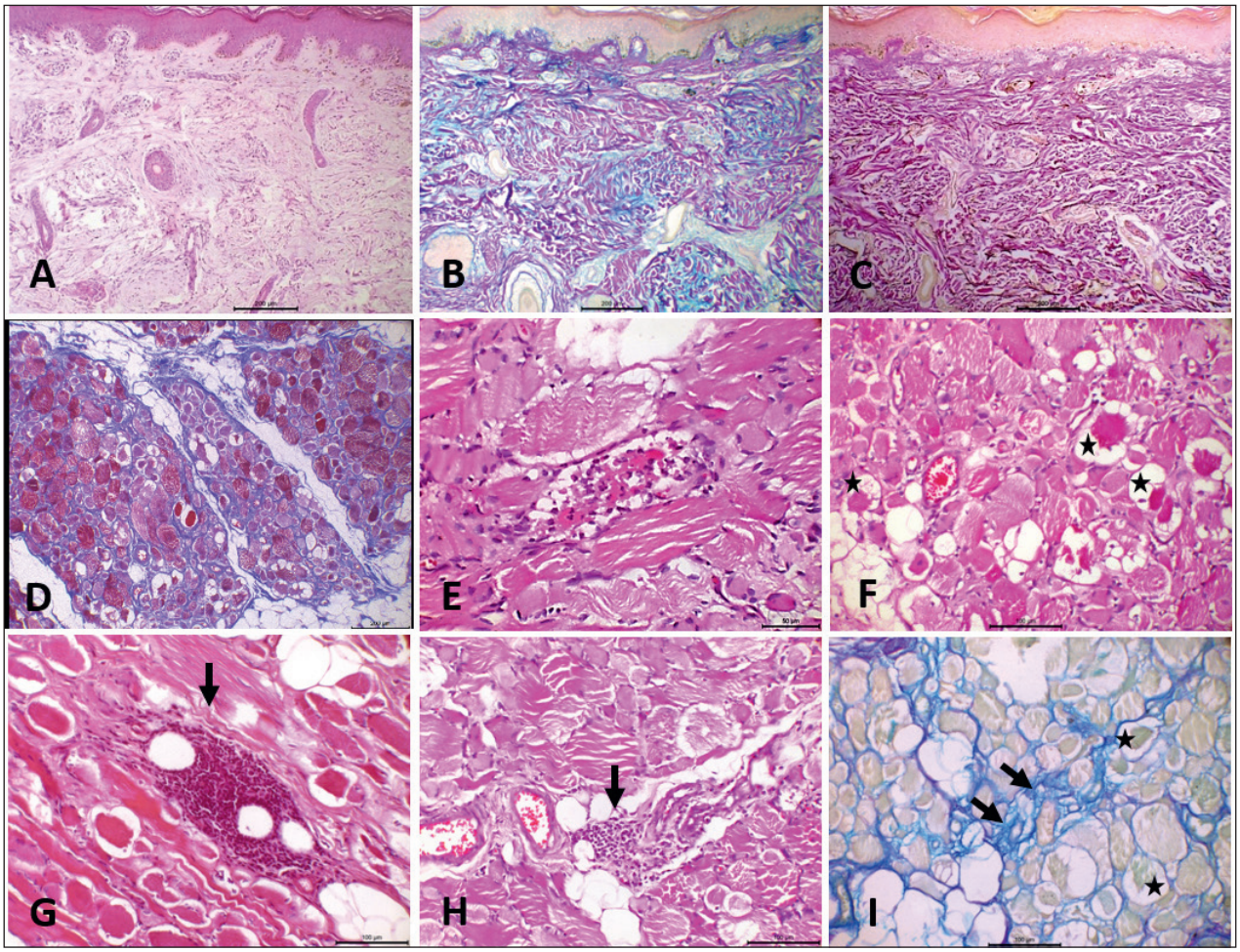

Figure 2. Skin biopsy shows dermal fibrosis with thick collagen bundles (A) separated by acid mucin (B) and associated with loss and fragmentation of elastic fibres (C). Left quadriceps muscle biopsy shows myopathic features like endomysial fibrosis (D), rounded fibers and myophagocytosis (E) with presence of intracytoplasmic vacuoles (F, *). In addition, focal endomysial (G, arrow) and perimysial (H, arrow) lymphocytic infiltration is also evident. Colloidal iron stain shows interstitial acid mucin deposition (I, arrow) without highlighting any vacuoles in myofibres (I, *). [A,E,F,G,H: Hematoxylin and Eosin; B,I: Colloidal iron; C: Verhoff van Gieson; D: Masson's trichrome. Magnification = Scale Bar A-D:200µm; F-I:100µm; E:50µm].

sociated myositis and dermatomyositis (9). The diagnosis in this situation was clinched by the accurate characterization of the skin lesions. The distribution of skin lesions in the mid-back and posterior auricular region differentiates scleromyxedema from scleroderma (5). The lesions also lack the distinctive distribution, erythema, and photosensitivity of dermatomyositis rash (4). In scleromyxedema, dysphagia results from oesophageal hypomotility and hoarseness (9) and recurrent aspiration from decreased laryngeal and vocal cord mobility (10).

Muscle pathology in scleromyxedema myopathy commonly reveals vacuolar degeneration of myofibres (11) and inflammatory myopathy (12) either in isolation or in combination (4). Other associated findings include varying degree of myophagocytosis, necrosis, regeneration, fiber splitting and internalization of nuclei. Despite the myofibres exhibiting large vacuoles, it is a rarity to demonstrate mucin deposition in skeletal muscle (11). The absence of deposits inside vacuoles has been reported in dermatomyositis, sarcotubular myopathy and scleromyxedema associated myopathy (4).

The treatment for scleromyxedema is impeded by the lack of clarity regarding the pathogenesis. The two successful modalities include immunotherapy and treatment directed against the paraproteinemia. They provide symptom control and limit progression, but the disease tends to relapse on cessation of therapy. The treatment of choice for cutaneous and extracutaneous disease is high

dose IVIg (6, 13) which usually provides excellent improvement. Long-term therapy is often required to sustain remission.

Second line therapy includes thalidomide with corticosteroids, but the response to steroid is usually partial (1). In severe and refractory cases, autologous hematopoietic stem cell transplantation, bortezomib, and melphalan have been tried (13). Mortality results from severe extracutaneous disease such as dermato-neuro syndrome, mucinous cardiomyopathy, and hematological malignancy (1).

In conclusion, scleromyxedema-associated myopathy is a rare disease which can masquerade as idiopathic and connective tissue disease-associated inflammatory myopathies. The clinical characterization of the skin lesions is key in suspecting the diagnosis. Though aggressive therapy provides disease control, the prognosis remains guarded due to the systemic complications and high relapse rate.

Conflict of interest

The Authors declare to have no conflict of interest.

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Modified Atkins ketogenic diet improves heart and skeletal muscle function in glycogen storage disease type III

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Glycogen storage disease type III (GSDIII) management in adult patients includes a high-protein diet with cornstarch supplementation to maintain a normal level of glucose in the blood. This regimen can prevent hypoglycaemia but does not seem to improve skeletal muscle and heart function. A 34 years-old patient with GSD IIIa with hypertrophic cardiomyopathy was then treated with a modified Atkins ketogenic diet. After 12 months of treatment ejection fraction raised from 30 to 45%, liver enzymes were reduced and CK plasma level dropped from 568 to 327 U/l. Physical activity increased from about 1300 to 2800 steps per day and health-related quality of life assessment ameliorated. An increase in uric acid triglycerides plasma level was observed. This data obtained in an adult patient confirm previous reports evidencing the effectiveness of ketogenic diets in improving cardiac and muscular manifestations in children with GSDIII.

Key words: glycogen storage disease type III, ketogenic diet, hypertrophic cardiomyopathy

Introduction

Glycogen storage disease type III (GSD-III), also known as Cori's disease, is a rare, autosomal recessive disorder of metabolism due to the deficiency of glycogen debranching enzyme. GSD types IIIa and IIIc mainly affect the liver and muscles, while GSD types IIIb and IIId typically affect only the liver (1, 2). The abnormal accumulation of limit dextrin results in frequent hypoglycaemia and both striated muscle and liver symptoms. In childhood, the phenotype is mainly characterized by hepatomegaly, short stature, hypoglycaemia and minimal skeletal muscle involvement that can worsen in adulthood. Heart involvement is rare in GSD3 children but in adult patients hypertrophic cardiomyopathy may happens.

Currently, the only treatment to limit glycogen storage is the diet. High-carbohydrate diet prevent fasting hypoglycemia but increases glycogen storage and does not slow the progression of cardiac and muscular manifestations. Ketogenic diets (KDs) are high-fat diets with an important carbohydrate reduction. Classic KDs are typically composed of a 4:1 or 3:1 ratio of fat (in grams) to protein plus carbohydrates (in grams). To improve compliance, other types of KDs have been proposed. Modified Atkins diet (MAD) is a high-fat and high-protein diet providing up to 20 g carbohydrate per day which is roughly equivalent to a ratio of 1-2:1 of fat to protein plus carbohydrates and does not require weighing of food portions (3). In this paper we report a case of a adult patient with GSD-IIIa treated with MAD.

Patient and methods

We observed a 34-year-old patients who was diagnosed GSD IIIa at the age of 9 months. He showed motor retardation, myopathy and hepatomegaly and then high-carbohydrate diet supplemented with uncooked cornstarch over night was started. At the age of 15 he was diagnosed with liver cirrhosis. Muscle involvement worsened with age causing distal weakness and exercise intolerance. Atthe age of 25 years hypertrophic cardiomyopathy was find and a high-protein diet providing 2.5 g protein/kg/d was initiated. At presentation to our outpatient clinic, cardiac function and general clinical picture had worsened despite the dietary treatment. Cardiac ultrasound showed increased left ventricle size, increase of parietal and septum thickness with diffuse hypokinesia. Ejection fraction was 30% and the patient had underwent diuretic therapy with furosemide, 300 mg/d, metolazone, 5 mg/d and spironolactone, 100 mg/d. Given the

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lack of efficacy of previous dietary approaches, a MAD with carbohydrates limited to 20 g per day was started. Food rich in fat and in protein like meat, fish, eggs, nuts were allowed ad libitum. Olive oil and medium chain triglycerides (MCTs) were recommended as seasoning fats. Vitamins and minerals were supplemented according to dietary recommendations. Uncooked cornstarch was prescribed only in case of hypoglycaemia.

Clinical and biological assessments took place before the start of the diet and every three months including: body weight, fasting plasma level of glucose, lactate, urea, beta2-microglobulin, creatinine, creatine kinase (CK), CK-muscle/brain (MB) mass and CK activity, uric acid, folates, vitamin B12, 25 OH-vitamin D, parathyroid hormone (PTH), N-terminal pro b-type natriuretic peptide (NTproBNP), plasma lipid profile, liver enzymes, echocardiography and liver ultrasonography. Urinary ketones were measured once daily by semi-quantitative test strips. To assess the amount of physical activity the daily step count was evaluated using a pedometer (Garmin Vivofit 3).

We assessed the patient's health-related quality of life (HRQoL) through the Italian version of the Short Form Health Survey (SF-36) questionnaire (4). The SF-36 contains eight domains;four domains assess the physical component (PC) of HRQoL (physical functioning, physical role, body pain, and general health) and four domains assess the mental component (MC) of HRQoL (vitality, social functioning, emotional role, and mental health). A score of 50 is regarded as the normal. For individual results, T-scores < 45 indicate impaired functioning in the domain.

Results

After 12 month of treatment MAD was well tolerated and the patient did not experience symptomatic hypoglycemia. Uncooked cornstarch or slow-release carbohydrates were never used during MAD. Semi-quantitative test strips showed high levels of KB in urines throughout the diet period. Body weight decreased from 67 to 64 kg. Echocardiography examination evidenced an increase of EF from 30 to 45%.CK plasma level dropped from 568 to 327 U/l, with a reduction both of CK-MB mass and CK-MB activity. NT-proBNP fell from 3010 to 2570 pg/ml. Furosemide was reduced from 300 mg/d to 75 mg/dand metolazone was suspended. Urea and beta-2 microglobulin decrease to almost normalize. Glomerular filtration, calculate according CKD-EPI creatinine equation (5) rise from 78 to 123 ml/min. Liver enzymes improved while C-LDL and triglycerides slightly increased (Table 1). Uric acid plasma level raised from 5.5 to 9.7 mg/dl and for this reason treatment with allopurinol 100 mg/d was started. Liver size did not change during the study.

Table 1. Biochemical and clinical patient data before and after 12 months of MAD treatment.

	T0	12 months
Weight (kg)	67	64
Plasma glucose g/dl	82	59
C-LDL g/dl	129	154
C-HDL g/dl	30	35
TG g/dl	111	134
Plasma uric acid g/dl	5.5	9.7
CK U/l	508	327
CK mass U/l	10.4	7.2
CK activity U/l	27	19
NT-proBNP pg/ml	3010	2570
AST U/l	122	87
ALT U/l	111	85
GGT U/l	133	38
LDH U/l	745	518
ALP U/l	234	129
PTH pg/ml	107	111
Plasma creatinine g/dl	1.2	0.7
Plasma urea g/dl	47	22
GFR ml/min/m^2	78	123
EF %	30	45
Steps /24h	1300	2800
Hr-QoL Physical component	42/100	58/100
Hr-QoL Mental component	48/100	52/100

Vitamin B12, 25 OH-vitamin D, parathyroid hormone (PTH) did not show notable variations.

Daily step count a increased from about 1300 daily steps before MAD to 2800 daily steps after 12 months of treatment. Before MAD HRQoL assessment showed a SF-36 PC subscore of 42/100 and a MC subscore of 48/100. After 12 months of MAD PS and MC subscores reached 58/100 and 52/100, respectively. The results are summarized in Table 2.

Discussion

In our patient MAD was able to induce and maintain ketosis without symptomatic hypoglycemia despite the very low intake of carbohydrates, probably due to gluconeogenesis from aminoacids and to higher plasma KBs availability. MAD significantly improved the clinical picture. The increase of FE and the reduction of heart failure laboratory tests evidenced the amelioration of cardiac function.

Renal function, which before MAD was impaired probably due to cardiomyopathy, improved and diuretics were reduced. The physical activity level assessed using the daily step count more than doubled compared

Table 2. Outcome data from case reports on KD in GSDIII for: author, patient number, age, length of follow-up, heart function and liver function effect, side effects.

Study	No. patients/age	Diet	Follow-up	Heart function	Liver function	Side effects
Valayannopoulos V [6] (2011)	1 (2 mo)	2:1 KD plus 3OHB	24 months	Improved	Stable	None
Brambilla A [5] (2014)	2 (5,7 y)	High-fat high-protein low-CHO	12 months	Improved	Improved	None
Mayorandan S [18] (2014)	2 (9,11 y)	MAD	32-26 months	Improved	Not reported	Transient asymptomatic hypoglycemia
Francini F	1 (34 y)	MAD	12 months	Improved	Improved	Increase of uric acid plasma level and C-LDL

to the levelmeasured before MADand the quality of life improved above all in the physical component. The rise in uric acid plasma level was the main metabolic adverse effect observed during the MAD period and it required a pharmacological therapy.. Lipid profile showed a little rise of triglycerides and C-LDL with a reduction of C-HDL/ C-LDL ratio. Worsening of cardiovascular risk is one of the main concern about the long-term treatment with KDs because of their high fat content. However, the studies employing long-term KDs demonstrated their relatively safety, the most common adverse effects including gastrointestinal disturbances, hyperlipidemia and hyperuricemia (6). Alterations of lipid profile does not necessarily lead to an increased cardiovascular risk because the low-carbohydrate diets cause an enlargement of LDL size and a reduction of more atherogenic small-LDL (7). Moreover, our patient was recommended to take olive oil and MCTs as seasoning fats as the former reduce cardiovascular risk, and the latter have not atherogenic effect and promote KDs synthesis (8). KD reduced transaminases plasma level and stabilized the liver size, likely due to a lesser limit-dextrin accumulation in hepatocytes.

This is the first case of adult GSDIIIa patient treated with KD. Previously, Dagli et al. (9) described a 22-year-old patient in whom a diet providing 30% of energy as protein with a low cornstarch supplementation (1.36 g/kg/d) improved cardiac function and normalized ventricular mass index. However, authors failed to report the exact amount of fat and CHO in the diet and the ketosis status was not evaluated.Valayannopoulos et al. (10) treated a 2-month-old infant with GSD III complicated by cardiomyopathy with a 2:1 KD supplemented with 3OHB up to 800 mg/kg/d. After 24 months the onset of this treatment, cardiomyopathy improved and motor development and somatic growth were normal. Brambilla et al. (11) reported a case of two siblings, 7- and 5- year-old, affected with GSD IIIa who developed a severe cardiomyopathy. They were first treated with frequent diurnal and nocturnal hyperproteic meals followed by uncooked cornstarch supplementation. Because a rapid worsening of cardiomyopathy a MAD-like diet (fat 60, 25%, carbohydrate 15%) was started at age seven for the girl and five for the boy. After 12 months exertion dyspnea improved, CK and NT-proBNP reduced and echocardiograms showed a marked improvement of cardiomyopathy.

Mayorandan et al. (12) treated two 9 and 11-year-old boys with GSD IIIa with a MAD (10 g carbohydrate per day, protein and fatty acids ad libitum) over a period of 32 and 26 months, respectively. At the end of observation CK plasma levels dropped and cardiac function markedly improved in the patient with severe cardiomyopathy. LDL-cholesterol levels were in the normal range and triglycerides slightly increased in one patient.

Increase of KBs plasma level during KDs could partly explain their efficacy in cardiomyopathy. Plasma KBs level raises in patients with severe heart congestive failure and the role of KBs as an alternative fuel in the failing human heart has recently been shown (13).

In conclusion, MAD showed a good efficacy in GSDIIIa treatment, improving physical activity, quality of life and overall cardiomyopathy, unlike the classic dietary approach. A part the low carbohydrates provision, the cases reporting a treatment with KDs in GSDIII differ with respect to the remaining dietary macronutrient. In our opinion, MAD has advantages over other diets because does not require calculating and weighing of protein and fat foods. Further studies are needed in order to investigate the long-term efficacy and safety of these diets.

Conflict of interest

The Authors declare to have no conflict of interest.

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CASE REPORTS

Rare variant in *LAMA2* gene causing congenital muscular dystrophy in a Sudanese family. A case report

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Congenital muscular dystrophies (CMD) are a heterogeneous group of disorders caused by mutations in musculoskeletal proteins. The most common type of CMD in Europe is Merosin-deficient CMD caused by mutations in laminin-α2 protein. Very few studies reported pathogenic variants underlying these disorders especially from Africa. In this study we report a rare variant (p.Arg148Trp, rs752485547) in *LAMA2* gene causing a mild form of Merosin-deficient CMD in a Sudanese family. The family consisted of two patients diagnosed clinically with congenital muscular dystrophy since childhood and five healthy siblings born to consanguineous parents. Whole exome sequencing was performed for the two patients and a healthy sibling. A rare missense variant (p.Arg148Trp, rs752485547) in *LAMA2* gene was discovered and verified using Sanger sequencing. The segregation pattern was consistent with autosomal recessive inheritance. The pathogenicity of this variant was predicted using bioinformatics tools. More studies are needed to explore the whole spectrum of mutations in CMD in patients from Sudan and other parts of the world.

Key words: congenital muscular dystrophy, Sudan, exome sequencing, novel variant

Abbreviations

LAMA2: laminin alpha-2

CMD: Congenital muscular dystrophy

Introduction

Congenital muscular dystrophies (CMD) are a heterogeneous group of disorders with phenotypic and genetic overlaps (1) caused by defects in structural proteins of skeletal muscle fibers e.g. Merosin, integrin, α-dystroglycan and others (2) molecular understanding of the congenital muscular dystrophies (CMDs. These diseases present commonly in early childhood with floppiness, progressive muscle weakness and skeletal deformities that severely impair the quality of life (3, 4). The heterogeneity in clinical presentation of CMD makes it difficult to estimate the burden of these diseases and certainly underestimates their prevalence (5).

Merosin-deficient CMD type 1A (MDC1A) is one of the most common forms of CMD accounting for 30-40% of cases in Europe (6). It is caused by mutations in laminin-alpha 2 (*LAMA2*) gene in chromosome 6q22-q23. Patients may present with muscle weakness, joint contractures, facial dysmorphism, peripheral motor neuropathy, epilepsy, developmental delay, elevated creatine kinase and white matter changes in brain MRI. The onset of symptoms, clinical presentation and partial or complete deficiency of laminin shows a broad range of variability in this type of dystrophy. Mild forms of CMD1A might resemble limb-girdle muscular dystrophy with peripheral motor neuropathy. Duplications, missense, nonsense and splice site mutations have been described. Thus, genetic analysis is necessary even in the presence of *LAMA2* protein in muscle biopsy (7).

Studies underlying genetics of CMD in general and the merosin deficient type (MD-CMD) in particular are relatively deficient especially in Africa where lack of expertise and high throughput technologies makes it difficult to discover novel candidate variants despite the well-

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known genetic heterogeneity of African populations (8) particularly in Africa, are important for reconstructing human evolutionary history and for understanding the genetic basis of phenotypic adaptation and complex disease. African populations are characterized by greater levels of genetic diversity, extensive population substructure, and less linkage disequilibrium (LD. In this article we report a rare variant (p.Arg148Trp, rs752485547) in *LAMA2* gene causing a mild form of Merosin-deficient CMD in a Sudanese family.

Materials and methods

Case presentation

Two Sudanese patients born to first degree consanguineous parents diagnosed with CMD since childhood were investigated. Patient 1 (the proband) was diagnosed at age 3 years. Patient 2 was diagnosed at age 4 years. Both patients presented with poor motor development, proximal muscle weakness, hypotonia and recurrent parasthesia but normal intellectual development. Patient 2 also suffered from epilepsy. There was a positive family history (a maternal uncle who passed away from the disease at age 36). The serum of the two patients showed elevation in CK level (120 units/ml, Normal: < 60 units/ml) and nerve conduction studies showed evidence of peripheral neuropathy. Patients refused to take a muscle biopsy for immunohistochemistry.

Five mL of peripheral venous blood was obtained from both patients, the parents, and two healthy siblings. Genomic DNA was extracted using guanidine chloride method as described in (9).

Whole exome sequencing

Exome sequencing was performed for two patients and a healthy elder sibling. The genomic DNA samples were enriched using TrueSeq library preparation kit v3 targeting a total length of 45Mb of the human coding exons. The samples were paired-end sequenced on an illumina HiSeq 2000 platform. The sequencing service was provided by Macrogen Inc. (Korea). The trimmed reads were aligned to the human genome assembly hg19 using bwa v0.7.12 (10). The duplicates were removed using samtools v1.2 (11). The alignment metrics were collected using Picard (http://broadinstitute.github.io/picard/). The percentage of the mapped unique reads was around 90%. The mean target coverage ranged around 90x for the three samples with ~ 90% of target bases covered at least 10x and 85% covered at least 20x. The variant calling was performed jointly for the three samples using freebayes v0.9.21 (12). The VCF file was annotated using VEP v84 (13) and loaded to Gemini v0.17 (14), then filtered for rare (ExAC MAF < 0.01) potentially pathogenic variants (CADD score > 10) that follow an autosomal recessive inheritance pattern (15, 16). A total of four shared runs of homozygosity (ROH) were identified using Homozygosity Mapper (17). We prioritized the variants that have a loss of function effect (stop-effect, frameshifts, splice-sites) or a damaging effect (both PPh2 damaging prediction and SIFT deleterious prediction) that are located in one of these homozygous run. This strategy effectively narrowed down the number of candidate variants to a single variant.

Sanger sequencing

For confirmation of genotypes, we performed Sanger sequencing for the candidate variant in the proband, his parents and a healthy sibling. Unfortunately, patient 2 passed away at age 36 years before the confirmation of his genotype through Sanger sequencing. Forward and reverse primers were designed using primer 3 (18). The primers used were: F 5' TCCCTAGGTGTTCCAGATCG, R 5' TTGTAAAGCGTTAGGCACTCC using the following PCR conditions: initial denaturation at 94°C for 2 minutes, followed by 35 cycles of 93°C for 30 seconds, 58.5°C for 30 seconds and 72°C for 20 seconds, with a final extension at 72°C for 5 minutes, to yield a PCR product of 153 base pairs in length. The PCR product was sequenced by Macrogen Inc (Korea). The resulting DNA sequences were aligned to the reference *LAMA2* DNA sequence from NCBI database using Bioedit software (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). The reference sequence (NM_000426.3) has been used for *LAMA2* variants.

Results

Whole exome sequence analysis revealed a rare C to T substitution in *LAMA2* gene (rs752485547, p.Arg148Trp) shared by the two patients but not their healthy brother. The variant was confirmed using standard Sanger sequencing and segregation analysis confirmed an autosomal recessive inheritance pattern (Fig. 1). The parents had a heterozygous (carrier) genotype; the proband was homozygous for the mutant variant and their healthy sister had a homozygous wild type genotype.

Discussion and conclusions

In this article we reported an ulra-rare missense variant in *LAMA2* gene (NM_000426.3:c.442C>T) causing a mild form of congenital muscular dystrophy in a Sudanese family. The variant has no reported clinical significance so far. It has a minor allele count of 3

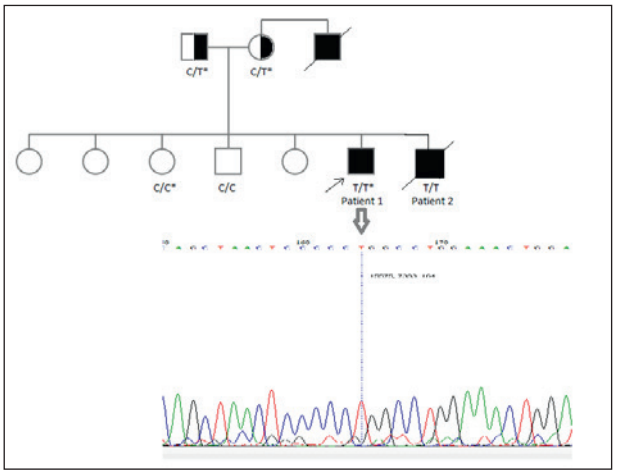

Figure 1. The pedigree of a Sudanese family with two patients with congenital muscular dystrophy. The arrow indicates the proband. The genotypes of the patients, parents and two healthy siblings are shown. The genotypes marked with (*) are detected or confirmed by Sanger sequencing. The electropherogram shows the result of Sanger sequencing in the proband.

(minor allele frequency of 0.00001218) according to the gnomAD database (14). It is moderately conserved among vertebrates (GERP rejected substitution score = 4.14) and predicted to be deleterious by more than five bioinformatic algorithms (SIFT, PPh2, Condel, Provean, FATHMM, MA) its amino acid change (p.Arg148Trp) is located in the Laminin N-terminal domain of *LAMA2* protein. *LAMA2* is important for cellular migration and organization during embryonic development (19). It acts as a mediator between extracellular and intracellular matrices (19). Changing one amino acid residue in a protein into another has variable impact on protein structure and function. Arginine is a positively charged amino acid and its replacement with Tryptophan; a hydrophobic amino acid and the largest in size will probably impairs the integrity of the protein especially in high and moderately conserved sites. According to the latest ACMG guidelines, this variant has at least two moderate (located in a mutational hot spot and/or critical and well-established functional domain, and at extremely low frequency in public databases) and two supporting (co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease, and multiple lines of computational evidence support a deleterious effect on the gene or gene product) criteria of pathogenicity (20). We therefore recommend the annotation of this variant as likely pathogenic.

Mutations in *LAMA2* gene causing CMD have been reported frequently in the literature from many parts of the world (21-29), especially in the past few years (22-26). However, studies are still lacking in Sub-Saharan Africa. Only one study has reported a novel mutation in *LAMA2* gene (NG_008678.1:g.437812T>C) in a Sudanese family (along with three other Saudi families) suggesting a founder haplotype in the region of Middle-East or Sudan (30). On the contrary, the current variant was seen in three different populations in gnomAD (once in each of the South Asian, Latino and non-Finish European populations). However, this part of the gene seems to represent a mutational hotspot. This variant (NM_000426.3:c.442C>T) is flanked by multiple rare and ultra-rare variants. A closely located variant (NM_000426.3:c.444dupG) is a known pathogenic insertion. Identification of causative mutations will certainly increase our understanding of the origin of disease-causing variants, molecular pathogenesis of the disease and perhaps new therapeutic modalities.

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Availability of data and materials

All data related to this article are available from the corresponding author upon reasonable request.

Author's contributions

MA and YB made substantial contributions to the design, administration and conceptualization of the study, interpretation of data and critically revising and drafting the manuscript. MK analyzed the data provided review and editing to the manuscript. MA, YB, MK, MO, OS, MS and MI participated in project conceptualization, data analysis and curation, review and editing. All authors approved the submission of the final manuscript and agreed to be accountable for all aspects of the work.

Ethics approval and consent to participate

This study was approved by ethical committee, Institute of Endemic Diseases, University of Khartoum, Sudan. Informed consent was obtained from each patient and family member before participation in the study.

Consent for publication

All participants (or parents/legal guardians in case of minors) provided consent for the publication of their clinical details.

Conflict of interest

The Authors declare no conflict of interest.

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Facio-scapulo-humeral muscular dystrophy with early joint contractures and rigid spine

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Early joint contractures in childhood or adolescence irrespective of muscle weakness are usually found in Emery-Dreifuss muscular dystrophy and collagen-VI related diseases and only rarely in the early stages of other progressive muscular dystrophies. We report a patient presenting severe elbow contractures and a rigid-spine since his early childhood without any evident muscle weakness, who was diagnosed with facioscapulohumeral muscular dystrophy later in life. This case is interesting since there has been no report, to date, of patients with a phenotype resembling facioscapulohumeral muscular dystrophy also in association with early and prominent elbow contractures and spinal rigidity, since childhood, resembling Emery-Dreifuss muscular dystrophy. Our case further confirmed the phenotypic variability often observed in carriers of *D4Z4* reduce allele, and highlights the complexity of a definitive diagnosis in these cases.

Key words: facioscapulohumeral muscular dystrophy, Emery-Dreifuss muscular dystrophy, joint contractures

Introduction

Early joint contractures and spinal rigidity, in childhood or adolescence, more prevalent than muscle weakness, are usually associated with Emery-Dreifuss muscular dystrophy (EDMD) where there is a scapuloperoneal pattern of weakness and heart involvement (1) and with collagen VI-related myopathies (2). In contrast, in progressive muscular dystrophies joint contractures usually appear late with advanced disease and are associated with severe weakness and reduced mobility. Nevertheless, prominent joint contractures, disproportionate to muscle weakness, have occasionally been reported in early stages of some limb-girdle muscular dystrophies (LGMD), usually calpainopathies (3, 4) or other rare myopathies such as LGMD1F, associated with mutations of the *TNPO3* gene (5), in BAG3-related myofibrillar myopathy (6), in myopathy with tubular aggregates related to *STIM1* gene mutations (7) and with recessive mutations of the *TTN* gene (8).

Facioscapulohumeral muscular dystrophy (FSHD) is one of the commonest muscular dystrophies (9) with disease onset ranging from childhood to late adulthood and typical clinical presentation involving facial muscles, shoulder girdle muscles, distal lower extremities muscles and later on proximal lower extremities muscles. There is a striking left to right asymmetry in muscle involvement and there is often severe weakness of axial muscles (9). Joint contractures appear late in the course of the disease and if prominent and early they set the diagnosis into question (10). We herein report the atypical phenotype of a patient diagnosed with FSHD who presented severe, early contractures and rigidity of the spine.

Case description

A 34-year old man, born from a non-consanguineous marriage, presented to our hospital reporting impairment in upper limb abduction, due to winged scapula, since the age of 27 years and subsequently a five-year slowly progressive difficulty in climbing stairs. He had no family history of neuromuscular disease.

On examination the patient had a waddling and stepping gait, bilateral scapular winging with impaired upper limb abduction, lumbar hyperlordosis and asymmetric lower facial muscle weakness. Asymmetric right sided atrophy of pectoralis and trapezius muscles was also evident. There was no weakness on upper limbs and on lower limbs there was severe distal weakness in the anterior leg compartment (tibialis anterior muscle graded 3/5 at the MRC scale), as well as weakness of the posterior thigh compartment (hamstrings graded 2/5 at the MRC scale). There was co-existing abdominal wall muscle weakness and a positive Beevor sign. Striking was the presence of severe bilateral elbow contractures (Fig. 1), that the pa-

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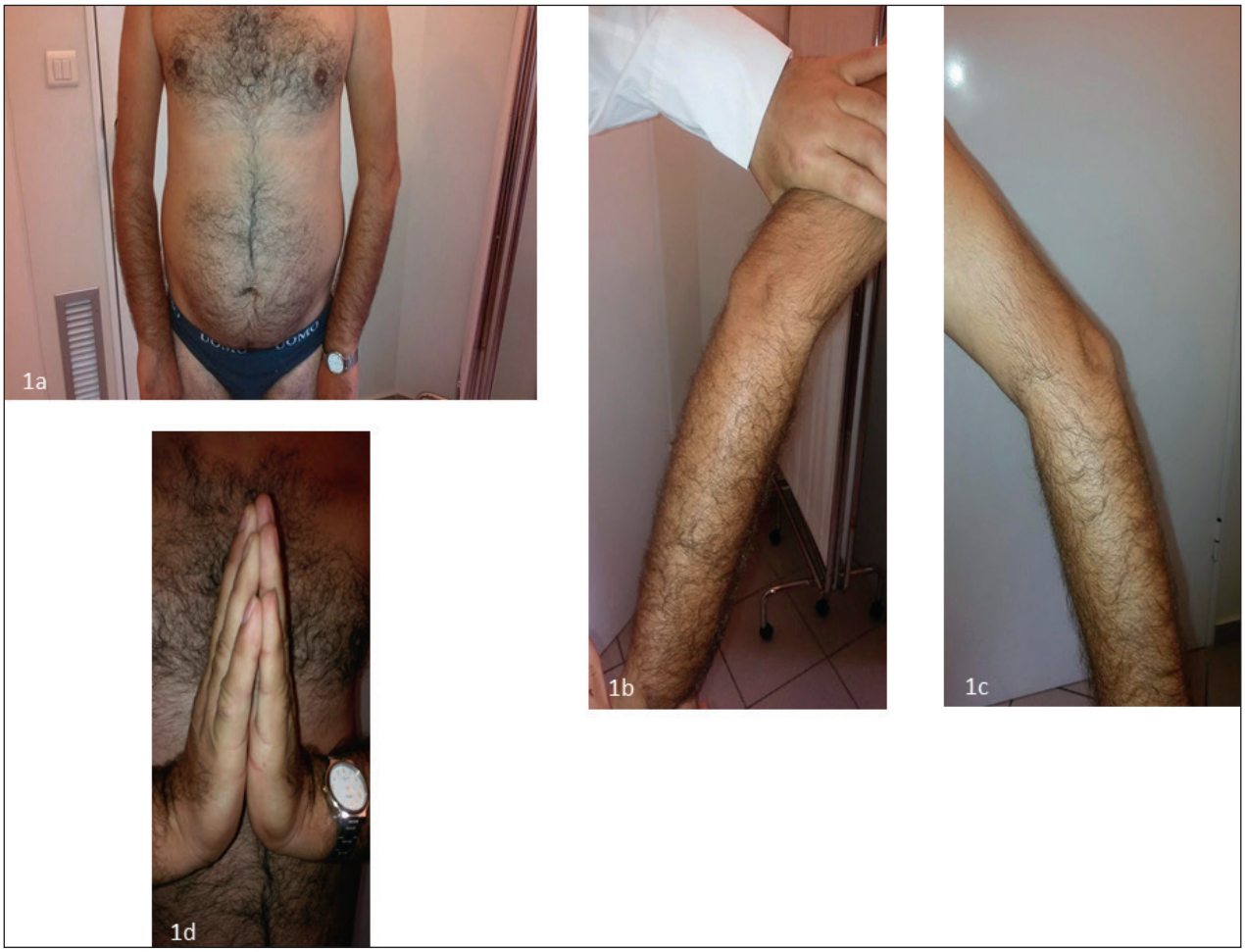

Figure 1. a) Bilateral elbows contractures; **b)** Right elbow contracture; **c)** Left elbow contracture; **d)** No "prayer sign": the patient can put the fingers close together with the elbows extended.

tient reported as being present since his early childhood, as well as of spinal rigidity, resembling an EDMD-like phenotype. Orthopedic framework at that time averred negative for an underlying bone/joint disease accounting for the contractures. His creatine kinase (CK) levels were 650 U/l (normal values 40-174 U/l). Electromyography showed myopathic traces in all examined muscles (defined as small amplitude, short duration, polyphasic motor unit action potentials, with early recruitment) as well as scarce spontaneous activity and rare myotonic discharges in biceps brachii and deltoid muscles, while nerve conduction studies were normal. A lower limb muscle MRI disclosed bilateral fatty infiltration of posterior thigh compartment as well as of tibialis anterior muscle in the lower legs.

Genetic testing was carried out for myotonic dystrophy type 1 and 2 due to the presence of myotonic discharges on electromyography and averred negative. Subsequent testing for restriction fragment at 4q35, revealed the presence of an allele with 10 *D4Z4* repeats, compatible with the diagnosis of FSHD with uncommon features (clinical category D1) (11). The patient refused to undergo further genetic testing for genes related to myopathies associated with early contractures (*LMNA, EMD, FHL1, TTN, COL6, CAPN3*) but agreed to a right tibialis anterior muscle biopsy. Muscle biopsy was processed as previously described (10) and revealed dystrophic features (muscle fiber necrosis and regeneration), no evidence of myofibrillar pathology or any abnormality in immunohistochemical studies.

Discussion

Facioscapulohumeral muscular dystrophy has a rather typical clinical presentation where joint contractures appear late in the course of the disease and if prominent and since the early stages, they set the diagnosis into question (12 13). In contrast to this concept we present the

interesting case of a young man diagnosed with FSHD, who presented with a scapuloperoneal syndrome with early and prominent elbow contractures and spinal rigidity, since childhood, resembling Emery-Dreifuss muscular dystrophy. The patient harbored a borderline contracted fragment of 10 *D4Z4* repeats in a permissive 4q35A chromosome, compatible with the diagnosis of FSHD (12). Longer *D4Z4* contractions are usually not de novo mutations (14) but often show reduce penetrance (15) probably explaining the lack of a positive family history in our patient. Unfortunately, refusal of other relatives to undertake genetic testing cannot exclude the presence of asymptomatic carriers among them. Our patient also reported early elbow contractions and rigid spine since early childhood, resembling an Emery-Dreifuss-like phenotype. Facioscapulohumeral muscular dystrophy "double trouble" condition patients are frequently reported (16) and such a "double trouble" with a co-existent myopathy associated with early contractures was not formally excluded by further genetic testing in our patient, since he refused. Nevertheless, there were no clinical or systemic manifestations reminiscent of another muscle disease. There was no family history of heart disease, nor did a meticulous cardiac evaluation disclose any heart involvement in him as it would have been expected in the case of EDMD (1). Muscular MRI didn't reveal the typical pattern we encounter in collagen VI related myopathies (17) and other typical clinical signs of collagenopathies such as keloids or prayer sign (Fig. 1d) were not found on clinical examination (2). As far as titin associated Emery-Dreifuss-like recessive muscular dystrophy it doesn't seem very likely since all previously reported cases had a childhood-onset disease (8). Calpainopathy seems not very probable since it doesn't present with a scapuloperoneal pattern of weakness and usually has higher CK levels (18) and on clinical examination we found no miosis or opthalmoparesis and the patient reported no myalgia features that are usually reported in *STIM1*-related myopathy (7, 19). In short of further genetic testing a tibialis anterior muscle biopsy was performed revealing neither myofibrillar pathology nor abnormalities in immunohistochemical staining suggestive of another coexisting myopathy that could account for his contractures.

Early contractures and spinal rigidity are usually not part of the typical clinical spectrum of FSHD (13). Nevertheless, atypical phenotypes associated with reduced *D4Z4* alleles, and completely different from the characteristic pattern of muscle involvement of the disease (12, 13) have already been described in carriers *D4Z4* reduced allele (16), especially, in patients with borderline contractions (20). Borderline *D4Z4* allele contractions have been associated with a broad myopathic spectrum, including FSHD with atypical phenotypes and it has been suggested that other factors besides *D4Z4* repeat number are involved in modulating disease penetrance, phenotype and severity in this borderline region (20). Our case confirms the large spectrum of phenotypes associated with *D4Z4* contraction, particularly with borderline alleles, highlights that early and prominent contractures and rigid spine can be found in a patient with FSHD and that if there are other signs of the disease, genetic testing should be performed. Whether this case represents a rare, underdiagnosed atypical phenotype of FSHD, or simply a "double trouble" with an underlying, second co-existing myopathic disorder needs to be further elucidated.

Conflicts of interest

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

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NEWS FROM AROUND THE WORLD

AIM

The XIX congress of the Italian Association of Myology will be held in Bergamo from 5 to 8 June 2019, organised by dr. Angela Berardinelli.

The Congress will be preceded by a satellite Symposium entitled: *Physical exercise: pros and cons for taking care of myopathic patients*. Those interested will find the program and the registration form in the "forthcoming meetings" section. All the readers all welcome.

MSM

The 14th Meeting of the Mediterranean Society of Myology (MSM) will be held in 2020. The date and location will be established shortly.

WMS

The 24th annual congress of the World Muscle Society will be held in the heart of Copenhagen in the old Tivoli Garden Concert Hall and adjoining buildings. Join WMS for the networking reception to be held on Tuesday 1st October in the theatre, Det Ny Teater, located a 5-minute walk from Tivoli gardens. This will follow the long tradition of WMS to facilitate networking and catch up on the latest developments in myology around the world during this 4-day meeting.

As usual, the meeting will be preceded by a teaching course, which will be held in Copenhagen on September 30th and October 1st 2019.

The Copenhagen Neuromuscular Center at the National Hospital, Rigshospitalet, led by John Vissing, will host and organise this meeting. Contributions about new advances across the neuromuscular field are very welcome. The main thematic topics that will be addressed in the plenary sessions will be:

1. Metabolic disturbances in neuromuscular diseases;
2. Extra-muscular manifestations in neuromuscular diseases;
3. Advances in the treatment of neuromuscular disorders;

Early bird registration is before Wednesday 8th May 2019 (midnight GMT).

FORTHCOMING MEETINGS

2019

February 5-6

Biospecimen Reasearch Symposium. Berlin, Germany. Information: website: *www.isber.org*

March 6-8

Advances in skeletal muscle biology in health and disease, University of Florida, Gainesville, FL, US. Information: website: *myology.institute.ufl.edu/conferences/muscle-biology-conference*

March 25-28

Myology 2019 AFM-Téléthon Scientific Congress in Myology, Bordeaux, France. Information: website: *www.afm-telethon.com*

April 4-5

11th Annual Neuromuscular Translational Research Conference, Newcastle, UK. Information: website: *www.ucl.ac.uk/cnmd/events*

May 4-10

American Academy of Neurology, 71st Annual Meeting, Philadelphia,PA, US. Information: website: *www.aan.com/conferences-community/upcoming-conference-dates*

May 7-10

ISBER 2019. Shangai, China. Information: website: *www.isber.org*

May 8-11

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

May 15-17

Annual Meeting of the French Society for Extracellular Matrix Biology. Reims, France. Information: *www.univ-reims.eu; comnco@comnconews.com*

May 2019

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

June 5

Physical exercise: pros and cons for taking care of myopathic patients. Satellite Symposiun of the 19th Annual Meeting of the Italian Association of Myology. Pavia, Italy. Information: website: *www.fclassevents.com*

June 6-8

19th annual Meeting of the Italian Association of Myology - Bergamo, Italy. Information: website: *www.fclassevents.com*

June 15-18

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

June 29 - July 2

European Academy of Neurology, 5th Congress, Oslo, Norway.Information: website: *www.ean.org/oslo2019/5th-Congress-of-the-European-Academy-of-Neurology-Oslo-2019.3649.0.html*

September 2-5

9th UK Nuclear Envelope and Chromatin Organization Meeting - 3rd International Meeting on Laminopathies. London, UK. Information: website: *www.laminopathiesmeeting2017.com*

October 1-5

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*

October 24-27

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

November 13-15

Third International Conference on Genomic Medicine (GeneMed-2019) in Baltimore, USA Information: website: *unitedscientificgroup.com/conferences/genemed*

December 9-11

6th TREA-NMD International Conference. Leiden, The Netherlands. Information: website: www.treat-nmd-conference.org

To be announced

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

2020

April 25 - May 1

American Academy of Neurology, 72nd Annual Meeting. Toronto, Ontario, Canada. Information: website: *www.aan.com/conferences-community/upcoming-conference-dates/*

June 6-9

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

September 30 - October 4

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

October 27-31

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

2021

September 21-25

26th Congress of World Muscle Society. Prague, Czech Republic, Information: website: *www.worldmusclesociety.org*

For application or renewal to MSM

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APPLICATION/RENEWAL FORM

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

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INSTRUCTIONS FOR AUTHORS

Acta Myologica publishes articles related to research in and the practice of primary myopathies, cardiomyopathies and neuromyopathies, including observational studies, clinical trials, epidemiology, health services and outcomes studies, and advances in applied (translational) and basic research.
Manuscripts are examined by the editorial staff and usually evaluated by expert reviewers assigned by the editors. Both clinical and basic articles will also be subject to statistical review, when appropriate. Provisional or final acceptance is based on originality, scientific content, and topical balance of the journal. Decisions are communicated by email, generally within eight weeks. All rebuttals must be submitted in writing to the editorial office.

On-line submission
Manuscript submission must be effected on line: **www.actamyologica.it** according to the following categories:
Original articles (maximum 5000 words, 8 figures or tables). A structured abstract of no more than 250 words should be included.
Reviews, Editorials (maximum 4000 words for Reviews and 1600 words for Editorials). These are usually commissioned by the Editors. Before spontaneously writing an Editorial or Review, it is advisable to contact the Editor to make sure that an article on the same or similar topic is not already in preparation.
Case Reports, Scientific Letters (maximum 1500 words, 10 references, 3 figures or tables, maximum 4 authors). A summary of 150 words may be included.
Letters to the Editor (maximum 600 words, 5 references). Letters commenting upon papers published in the journal during the previous year or concerning news in the myologic, cardio-myologic or neuro-myologic field, will be welcome. All Authors must sign the letter.
Rapid Reports (maximum 400 words, 5 references, 2 figures or tables). A letter should be included explaining why the author considers the paper justifies rapid processing.
Lectura. Invited formal discourse as a method of instruction. The structure will be suggested by the Editor.
Congress Proceedings either in the form of Selected Abstracts or Proceedings will be taken into consideration.
Information concerning new books, congresses and symposia, will be published if conforming to the policy of the Journal.
The manuscripts should be arranged as follows: 1) Title, authors, address institution, address for correspondence; 2) Repeat title, abstract, key words; 3) Text; 4) References; 5) Legends; 6) Figures or tables. Pages should be numbered (title page as page 1).
Title page. Check that it represents the content of the paper and is not misleading. Also suggest a short running title.
Key words. Supply up to three key words. Wherever possible, use terms from Index Medicus – Medical Subject Headings.
Text. Only international SI units and symbols must be used in the text. Tables and figures should be cited in numerical order as first mentioned in the text. Patients must be identified by numbers not initials.
Illustrations. Figures should be sent in .jpeg or .tiff format. Legends should be typed double-spaced and numbered with Arabic numerals corresponding to the illustrations. When symbols, arrows, numbers, or letters are used to identify parts of the illustrations, each should be explained clearly in the legend. For photomicrographs, the internal scale markers should be defined and the methods of staining should be given.
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Standard journal article: Figarella-Branger D, Bartoli C, Civatte M, et al. Cytokines, chemokines and cell adhesion molecules in idiopathic inflammatory myopathies. Acta Myol 2000;19:207-8.
Books and other monographs: Dubowitz V. Muscle disorders in childhood. London: WB Saunders Company Ltd; 1978.
Please check each item of the following checklist before mailing:

- Three index terms, short title for running head (no more than 40 letter spaces) on the title page.
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- Summary (maximum 250 words).
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- References prepared according to instructions.
- English style.
- Patients in photographs not recognisable.