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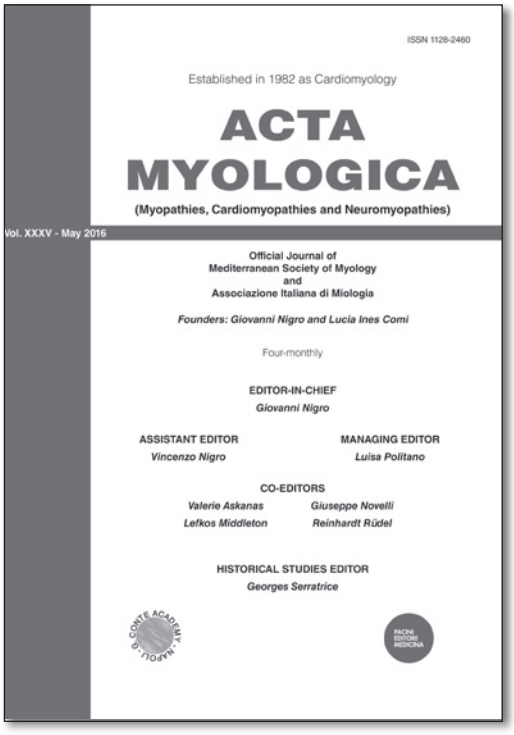

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EDITORIAL

Magnetic resonance imaging in muscular dystrophies

Muscular dystrophies present with a broad often overlapping diagnostic spectrum which may ultimately require muscle biopsy.

Magnetic Resonance Imaging (MRI) is growing in popularity and becoming more and more followers, because its capacity to reveal characteristic findings which can address the diagnosis, aid in determining optimal biopsy sites and control therapeutic interventions.

Several papers published during the past few years have reported on the value of MRI in detecting patterns of muscle involvement in various muscular dystrophies and other inherited myopathies. Such a technique provides a high soft tissue contrast allowing excellent assessment of striated muscles concerning shape, volume (hypotrophy, hypertrophy) and tissue architecture (1-4).

Because of the lack of ionizing radiation, MRI has become a valuable imaging method in children, although sometimes sedation might be necessary. Basically, MRI is performed as a multi-sequence imaging protocol including T1-weighted (T1W) and T2-weighted (T2W) (turbo) spin echo as well as fat-suppressed (short tau inversion recovery or spectral fat suppression techniques) T2-weighted sequences (T2WFS). The image acquisition is performed in the axial plane with a slice thickness of 5-7 mm. If necessary, additional images in other anatomical planes (coronal, sagittal) can be easily acquired. MRI can be performed and rated in a standardized manner suggesting a good inter-rater and intra-rater (during follow-up) agreement.

In the early stages of Duchenne Muscular Dystrophy (DMD), MRI shows an early involvement of the gastrocnemii (5, 6). An abnormal signal in the gluteus maximus and adductor magnus, followed by involvement of the quadriceps, rectus femoris, and biceps femoris, with selective sparing of the sartorius, gracilis, semitendinosus, and semimembranosus is observed in the advanced stages (7-9).

A distinct pattern on muscle imaging characterized by prominent involvement of the gluteus maximus and medius, adductor magnus, biceps femoris long head, semi-membranosus and vasti was also observed in individuals with Becker Muscular Dystrophy (BMD), a milder form secondary to mutations in DMD gene (10, 11).

A specific pattern of muscle fatty replacement and atrophy, particularly in upper girdle muscles have been reported in Facio-Scapulo-Humeral-Dystrophy (FSHD) patients. The most frequently affected muscles, including paucisymptomatic and severely affected patients, were trapezius, teres major and serratus anterior, in a characteristic asymmetric fashion (12).

In some forms of limb-girdle muscular dystrophies such as dysferlinopathies and anoctaminopathies, due respectively to mutations in DYSF and ANO5 genes, a predominant fatty degeneration of the gluteus minimus muscle and of the posterior segments of the thigh and calf muscles, with sparing of the gracilis muscle, was observed (13).

MRI was proved useful also in patients with oculopharyngeal muscular dystrophy (OPMD), where it revealed distal lower legs more severely fatty replaced than the thigh muscles. Soleus and long head of the biceps femoris was severely involved in all patients, whereas popliteus, gracilis and short head of biceps femoris were almost completely spared, even in advanced stages (14).

A few recent studies have reported muscle MRI findings in the most common forms of Congenital Muscular Dystrophies (CMD), those secondary to mutations in the collagen VI genes (Ullrich CMD), though no systematic studies have evaluated muscle MRI in all of the genetically recognized forms of CMD. Patients with Ullrich CMD show diffuse involvement of all the posterior and lateral muscles of the thigh with selective sparing of the sartorius, gracilis, and adductor longus, and often the rectus femoris (15-18). These signs have a significant overlap with those observed in Bethlem myopathy, a milder dominant condition allelic to Ullrich CMD.

It has been shown that muscle MRI is able to distinguish the various forms of congenital muscular dystrophy, despite a significant clinical overlap. For example,

patients with RSMD1, a condition secondary to deficiency in selenoprotein 1, who also have rigidity of the spine, early respiratory involvement, and normal or only mildly elevated CK, present a peculiar MRI muscle involvement (17, 18).

In the present issue we publish the article review of Díaz-Manera et al. on muscle MRI pattern in various forms of muscular dystrophies and the paper of Maggi et al. on the usefulness of MRI in muscle channellopathies.

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ORIGINAL ARTICLES

Muscle MRI in muscular dystrophies

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Muscle MRI has become a very useful tool in the diagnosis and follow-up of patients with muscle dystrophies. Muscle MRI provides us about many aspects of the structure and function of skeletal muscles, such as the presence of oedema or fatty infiltration. In the last years many reports have described the particular muscles that are involved in these muscle disease. This knowledge can facilitate the diagnosis in many cases. In the present paper we review the main changes observed in muscle MRI of patients with muscle dystrophies

Key words: Limb-girdle muscular dystrophies; muscle MRI

Introduction

Muscle MRI has become a useful tool for diagnosis and follow-up of patients with limb girdle muscle dystrophies (LGMD). Muscle MRI provides information on different aspects of muscle structure and function of muscles of the body. The sequences available allow to identify fatty infiltration (T1 or 3-point Dixon sequences) or oedema (STIR or T2) in the muscles (1). Therefore, muscle MRI is useful to select a muscle for the biopsy in patients without clear muscle weakness or in patients with a severe degree of muscle atrophy, in which muscle tissue can be completely substituted by fat. Moreover, because MRI does not use radiation, it is a good technique to follow-up the progression of the disease in patients (2). There is a growing number of evidences demonstrating that the degree of muscle fatty infiltration observed in muscle MRI correlates with the muscle strength and functional status of patients with LGMD (2-4). For this reason, muscle MRI is being progressively included as a primary endpoint in clinical trials of patients with muscle dystrophy (5, 6).

It is well known that every single muscle dystrophy induces fatty infiltration of a particular group of muscles. This fact allows investigators to design diagnosis algorithms based on the MRI findings (7). However the number of reports describing the changes observed in every muscle disease has grown in the last years and a review of the main findings observed in every disease is needed. Our aim is to describe our protocol to perform muscle MRI in patients with muscle dystrophy and to summarize the main findings of MRI studies in LGMD.

Methods

Muscle MRI protocol

Good quality Muscle MRI studies can be conducted in a 1.5 Tesla MRI scanner. In our center, we are equipped with a Phillips Achieva XR Magnetic Resonance System (Philips, Eindhoven, Netherlands). Our MRI system has a moveable tabletop that allows performing whole body studies in a short period of time (less than 30 minutes) without relocating the patient. Our standard protocol includes T1 and STIR sequences. T1 weighted spin-echo sequence obtains axial and coronal images from the head to the toes using the following parameters: repetition time: 300 ms, echo-time: 10 ms and thickness 10 mm. Short-time inversion recovery (STIR) sequences obtains coronal images using the following parameters: repetition time 2,500-3,500 ms, echo time 60 ms, inversion time 150 ms in 10 mm slices.

We quantify the degree of muscle fatty infiltration using the Mercuri scale modified by Fischer et al in 2008 that has been used to analyse both muscle MRI and CT scans (8) (Fig. 1):

- *Normal muscle appearance:* 0 points.
- *Mild involvement:* traces of increased signal intensity on the T1-weighted MR sequences: 1 point.
- *Moderate involvement:* increased T1-weighted signal

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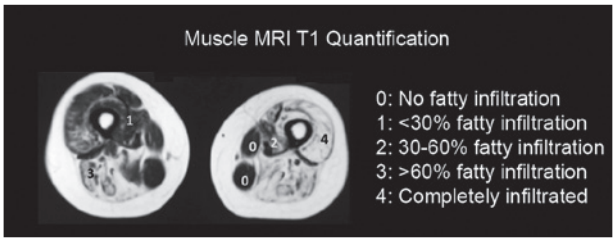

Figure 1. Diagram showing our quantification method of the fatty infiltration present in T1 sequences. In the image, a thigh slice is quantified using the Mercuri scale modified by Fischer.

intensity with beginning confluence in less than 50% of the muscle: 2 points.

- *Severe involvement:* increased T1-weighted signal intensity with beginning confluence in more than 50% of the muscle: 3 points.
- *End-stage appearance*: entire muscle replaced by increased density of connective tissue and fat: 4 points.

Our protocol to analyze a whole body muscle MRI is the following: first we quantify the degree of fatty infiltration of the muscles of all body. Then, we fill out a table containing the name of the muscles with the value of the quantification. In this way, we are able to easily identify which are the most and the less involved muscles. Finally, we compare the results with the patterns already published in the literature to identify a probable diagnostic.

We use 3-point Dixon sequences for investigation purposes only, for example in natural history studies or in clinical trials. 3-point Dixon studies require a specific software to quantify the amount of fat and water per pixel in every muscle (9). Although the process of analysis of the image is longer than with T1 sequence, the results are more reliable and can be compared from one center to the other.

We use CT scan to study patients in which muscle MRI is contraindicated, for example in patients with a metallic prosthesis, a pacemaker or with claustrophobia. In these cases, whole body CT scan is performed using the following parameters: 140 kV and 120-350 mA. Section thickness is of 1.25 mm, with a section interval of 0.6 mm. The axial images are 5 mm thick, with an increment of 5 mm.

Muscle MRI in dystrophinopathies

Mutations in the dystrophin gene produce different phenotypes such as Duchenne muscle dystrophy and Becker muscle dystrophy. The MRI pattern of muscle atrophy has been well investigated both in patients with

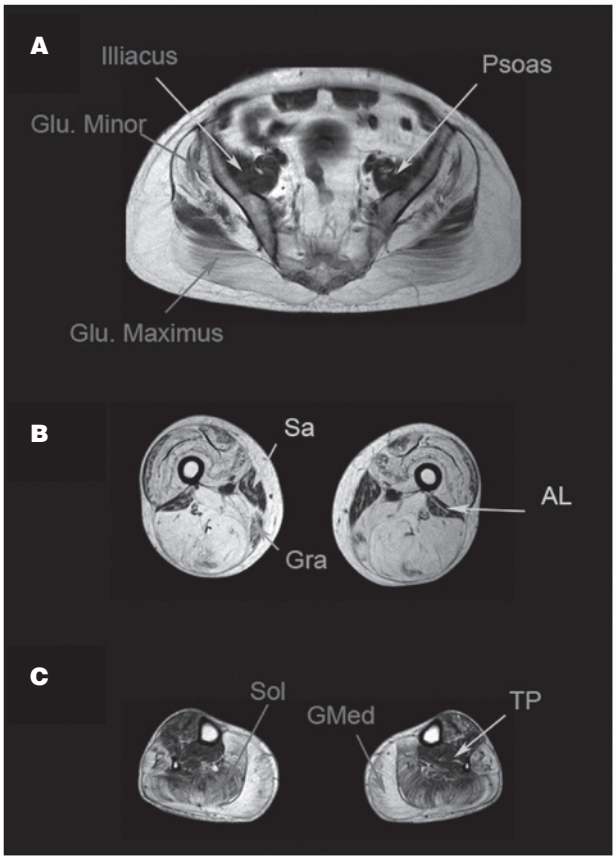

Figure 2. Muscle MRI of a Becker muscle dystrophy patient. A) Pelvic image shows atrophy of glutei muscles (Gluteus minimus and maximus are shown in the image). Psoas and illiacus muscle are usually spared until late stages of the disease. B) Image of the thigh shows a complete atrophy of all muscles, except for sartorius (Sar), gracillis (Gra) and adductor longus (AL). C) Image of the legs showing a severe atrophy of gastrocnemius medialis (GMed) and soleus (Sol). In this patient, tibialis posterior (TP) was not involved.

Duchenne and Becker muscle disease (4, 10, 11). In both cases the pattern is very similar. Studies have been focused on pelvic, thigh and leg muscles (Fig. 2). In general muscle atrophy is symmetric. The muscles more commonly involved are:

a. Pelvis: *Gluteus maximus* and *medius* are involved from the onset of the disease and become progressively atrophied until late stages of the disease when all the glutei muscles are atrophic. In this later stage *pso*as and *iliacus* muscles could also become atrophic. *Obturator internus* and *externus* are rarely involved.

b. Thighs: Muscles from the anterior and posterior compartment of the thigh are involved in most of the patients, including *adductor major*, *biceps*, *semi-*

membranosus, *semitendinosus* and the *vasti*. In contrast *sartorius*, *gracilis* and *adductor longus* are not involved until later stages of the disease.

c. **Legs:** There is a common involvement of both *gastrocnemius*, *soleus* and *peroneus* muscles. In contrast *tibialis posterior* is not commonly involved trough all the progression of the disease.

Keys for the diagnosis

In more than 90% of the patients there is a moderate to severe atrophy of *gluteus maximus*, *medius*, *semimembranosus* and the *vasti* (4).

Mild involvement or not involvement of *psoas*, *iliacus*, *sartorius*, *gracilis* and *adductor longus*. To find a normal *adductor longus* among completely atrophic thigh muscles is a good clue for the diagnosis.

There is only one study that has analysed muscles of upper limbs in patients with Duchenne muscle dystrophy (12), showing a preferential involvement of *triceps*, *biceps*, *teres major* and periscapular muscles including *supraspinatus*, *infraspinatus* and *subscapularis.* In contrast, *deltoid* is usually not involved.

The pattern of muscle atrophy of symptomatic carriers of a mutation in the dystrophin gene is very similar to the one found in Becker muscle dystrophy (13). As we have just mentioned, a normal *adductor longus* is a clue for the diagnosis. Moreover, many of the symptomatic carriers have clear asymmetric changes that, when present, can easily guide the diagnosis.

Muscle MRI in autosomal dominant limb girdle muscle dystrophies (AD-LGMDS)

1. LGMD1A or Myotilinopathy

Patients with mutations in the *MYOT* gene share a common pattern of muscle fatty infiltration on MRI studies with other myofibrillar myopathies, such as the ones

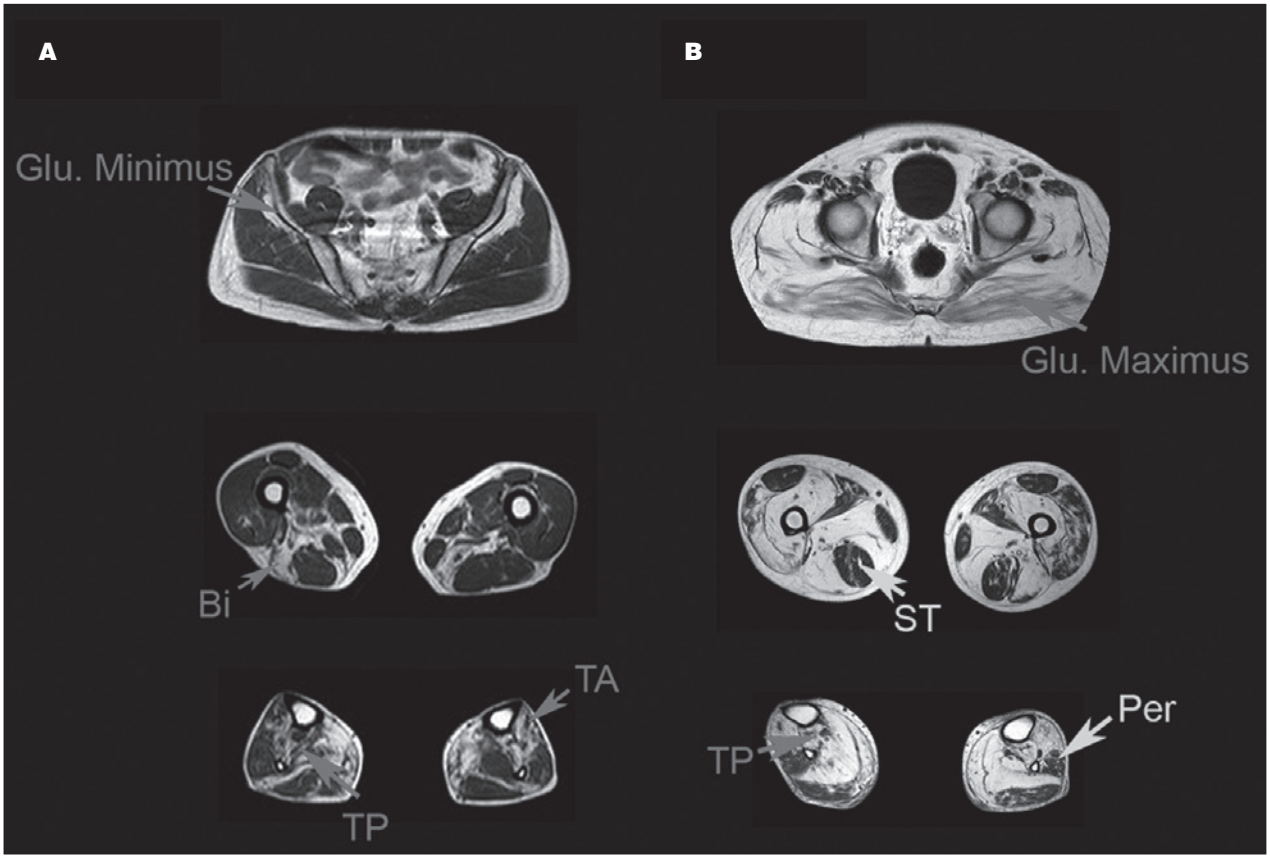

Figure 3. Muscle MRI of two patients with mutations in the MYOT gene. A). Patient with mild weakness: muscle MRI shows mild involvement of gluteus minimus, biceps (Bi), tibialis anterior (TA) and tibialis posterior (TP). B) Patient with severe weakness: muscle MRI shows involvement of all glutei muscles, although gluteus maximus is less involved than gluteus minimus or medius. In the thighs, semitendinosus (ST) is not involved. In contrast, there is a clear involvement of the posterior muscles of the thighs. Tibialis posterior (TP) is more atrophic than peroneus muscle (Per).

produced by mutation in the *ZASP* and the *FLNC* gene [8, 14, 15] (Figure 3). The muscles involved are:

a. Pelvis: Although there are not enough data to establish a common pattern, in our experience *gluteus maximus* is less involved than *gluteus medius* and *minimus*.

b. Thighs: In general, *adductor major*, *biceps* and *semimebranosus* are involved in all patients. *Vasti* muscles and *sartorius* can be also atrophic in many cases. In contrast *semitendinosus* is not involved until late stages of the disease.

c. Legs: There is a common involvement of *gastrocnemius medialis*, *soleus*, *tibialis anterior* and *posterior*. *Peroneus* muscles is not involved until late stages of the disease.

Keys for the diagnosis

To find a spared *semitendinosus* muscle associated to a severe atrophy of *biceps* and *semimembranosus* is a clue for the diagnosis.

Atrophy of *tibialis posterior* in a higher degree than *peroneus* muscle is also useful to identify this disease.

2. LGMD1B or Laminopathy

There are many publications describing the pattern of muscle involvement in patients with mutations in the *LMNA* gene (16-18]). The studies already published in adult patients have been focused on muscles of the lower limbs. The pattern seems to be homogeneous among patients and is characterized by a symmetric involvement of posterior muscles of the thighs and legs associated to a severe involvement of the paravertebral muscles (Figure 4). We have observed that this pattern is not different of the muscle involvement observed in patients with mutations in the *STA* gene (19). The muscles preferentially involved are:

a. Trunk muscles: Paraspinal muscles become involved from the onset of the disease, especially *multifidus* and *longissimus.* Different to other muscle diseases, such as Pompe disease, abdominal muscles are not commonly involved during the progression of the disease.

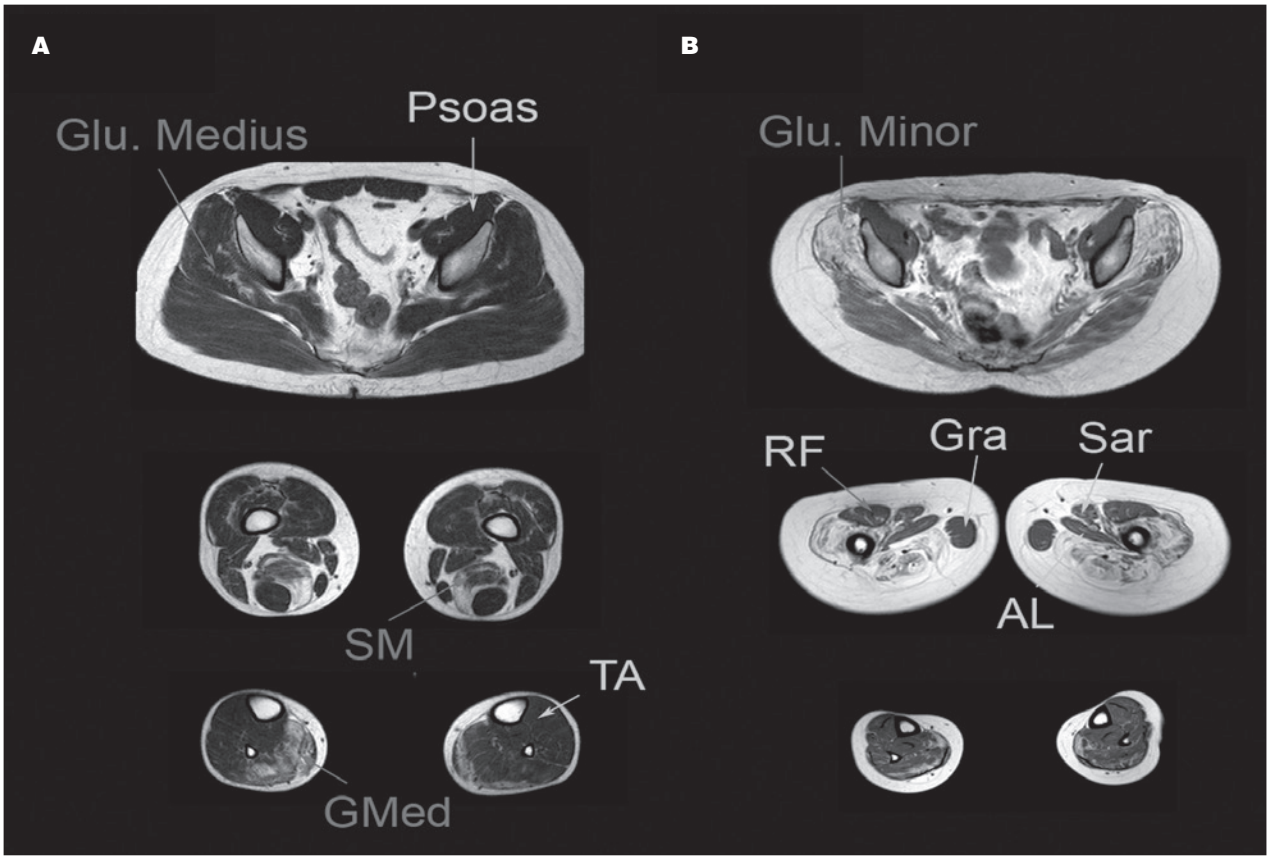

Figure 4. Muscle MRI of two patients with mutations in the LMNA gene. A) Patient with mild weakness. In this patient we observed fatty infiltration in the gluteus medius, semimembranosus (SM) and gastrocnemius medialis (GMed) muscles. B) Patient with severe weakness: fatty infiltration was observed in gluteus minimus and medius. All the muscles of the thighs were involved, except rectus femoris (RF), adductor longus (AL), sartorius (Sar) and gracillis (Gra) that were not atrophic.

b. Pelvis: *Gluteus minimus* and *medius* are involved in most of the patients, and in all the cases analysed by us these two muscles have a more severe degree of muscle atrophy than *Gluteus maximus*. *Psoas* muscle is rarely involved in these patients.

c. Thighs: The posterior muscles of the thighs, especially *semimembranosus*, the long head of *biceps* and the *adductor major* muscles are involved from the onset of the disease. In later stages *semitendinosus* and the short head of the *biceps* become also atrophic. Vasti muscles are involved in most of the patients: *vastus intermedius* is usually the first *vasti* muscle involved, followed by *vastus lateralis* and *medialis*. We have observed that the lateral part of the *vastus lateralis* is not involved until very late stages of the disease. *Rectus femoris* is normally not involved and becomes hypertrophic in many patients, as well as *sartorius* and *gracillis* muscles.

d. Legs: The posterior compartment of the legs is involved in all patients. Medial *gastrocnemius* is severely involved in most of the patients, associated in many cases to atrophy of *soleus* and *gastrocnemius lateralis*. The muscles of the anterolateral compartment are rarely involved during the progression of the disease.

Keys for the diagnosis

The combination of atrophy involving the anterior and posterior compartment of the thighs, associated to a severe atrophy of the posterior muscles of the legs and hypertrophy of *rectus femoris* is a very suggestive pattern of involvement in patients with mutations in the *LMNA* gene.

3. LGMD1C or Caveolinopathy

There is not enough information published of patients with caveolinopathy to establish a pattern of muscle involvement. We have studied patients with rippling muscle disease or isolated hyperckemia and the muscle MRI is normal in these cases.

5. LGMD1D

LGMD-1D is produced by mutations in the *DNABJB6* gene. There is only one paper published reviewing the radiological features of 23 patients with this disease (20). In the initial stage of the disease, the muscles more commonly involved were the *adductor major*, the *semimembranosus*, the *biceps* and the *soleus*. In a second stage, *gastrocnemius medialis* and *adductor longus* were also involved. In advanced stages, atrophy also involved *vastus medialis*, *laterallis* and *intermedius*. *Sartorius*, *rectus femoris*, *gracillis*, *gastrocnemius lateralis* and the antero-lateral compartment of the legs were not involved until late stages of the disease. The most characteristic feature in this disease is a preservation of *semitendinosus* until latter stages, as it happens in patients with mutations in the *MYOT* gene.

6. LGMD-1E or Desminopathy

Patients with mutation in the *DES* gene develop a myofibrillar myopathy. The radiological features are very similar to those found in patients with mutation in the *CRYAB* gene [8]. There are many reports describing the common features in these patients, although the studies have focused mainly on thighs and legs area (Fig. 5) (14).

a. Thighs: Fatty infiltration involving the *semitendinosus*, the *gracillis* and the *sartorius* muscles occurs from the onset of the disease. This pattern is very suggestive of desminopathy and is not commonly observed in other diseases. Latter on, the *vasti* can also be involved.

b. Legs: *Peroneus* is the most commonly involved muscle in these patients, associated commonly to atrophy of the posterior muscles of the legs.

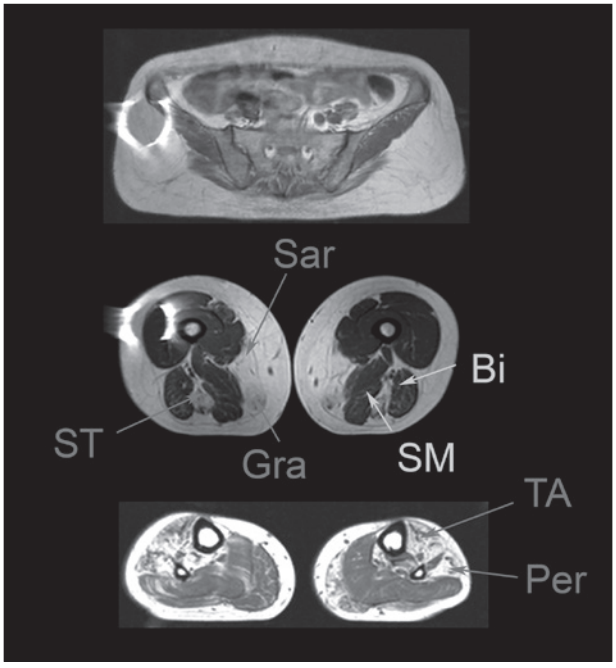

Figure 5. Muscle MRI of a patient with mutation is the DES gene. Muscle MRI shows a preferential involvement of semitendinosus (ST), sartorius (Sar) and gracillis (Gra) muscles, while in contrast semimembranosus (SM) and biceps (Bi) are not involved. In the legs, there is an involvement of tibialis anterior (TA) and peroneus muscles (Per). Courtesy of Dr. Giorgio Tasca.

Muscle MRI in autosomal recessive limb girdle muscle dystrophies (AR-LGMDS)

1. LGMD2A or calpainopathy

There is an important variability in the patterns described in patients with mutations in the *CAPN3* gene (21-23). However there are some common traits that can be found in most of the patients (Figure 6). The common muscles involved in this disease are:

a. Trunk and pelvis area: Paravertebral muscles are involved in most of the patients from the onset of the disease. In contrast, abdominal muscles are rarely involved and they are not affected until late stages. The glutei muscles are also involved from the onset, especially *glutei medius* and *minimus*, while *glutei maximus* tend to be involved in more advanced patients (24). *Psoas* muscles is not commonly involved until late stages.

b. Thighs: *Adductor major* and *longus* and *semimembranosus* are the first muscles involved. Later on the progression of the disease, the rest of the muscles of the posterior compartment of the thigh become involved. In many patients there is a clear difference between the anterior muscles (normally spared) and posterior muscles (atrophic) of the thighs. However, involvement of the anterior compartment is not uncommon, and is frequently observed in medium and advanced patients. The *vasti* muscles are involved: in a first step the *vastus intermedius* and then the *vastus medialis* and *lateralis*. *Rectus femoris* can also be involved in many patients. In general, *sartorius* and *gracillis* are not involved until very late stages of the disease.

c. Legs: One of the clues for the diagnosis is the common involvement of *gastrocnemius medialis* and *soleus* from the onset of the disease. However, as the disease advances, atrophy involves the *gastrocnemius lateralis*, the *peroneus* and the *tibialis anterior*.

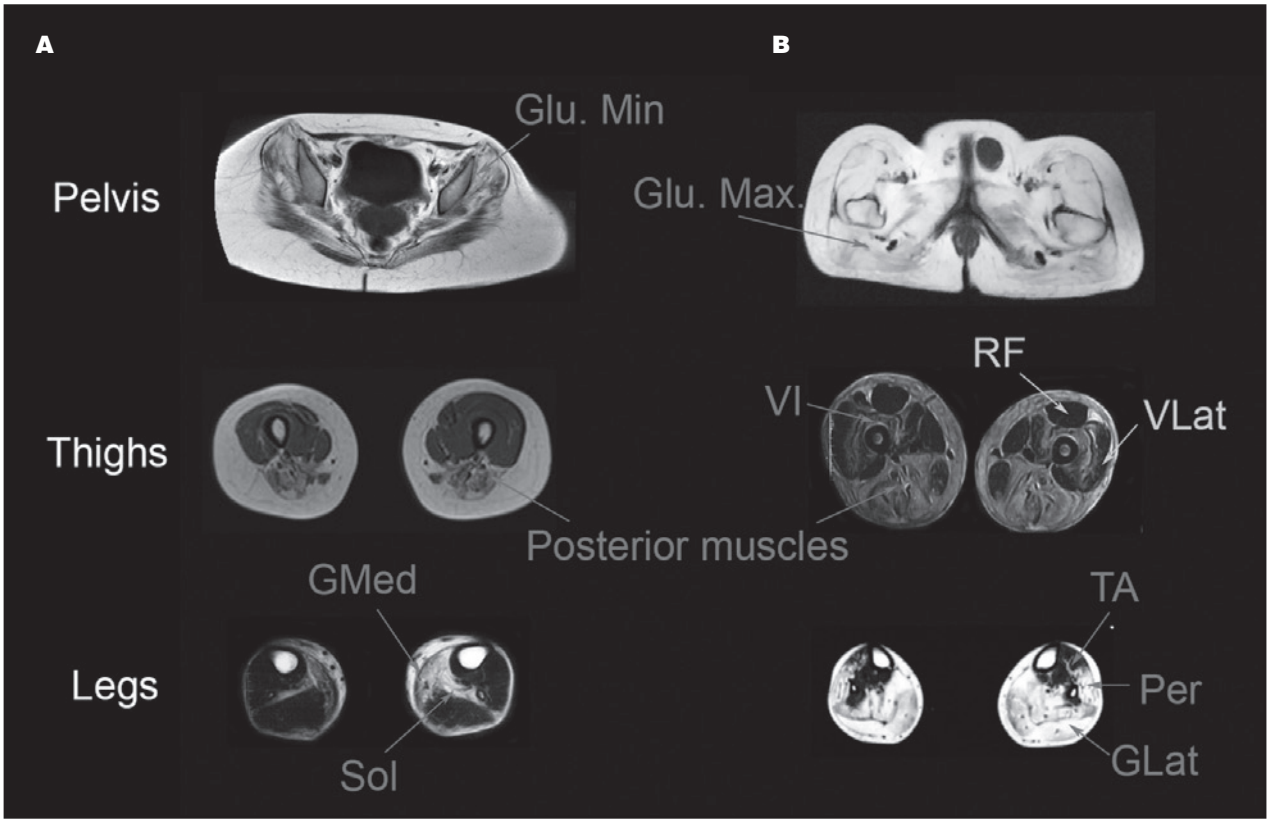

Figure 6. Muscle MRI in patients with mutations in the CAPN3 gene. Gluteus minimus and medius tend to be more involved than gluteus maximus (A) in most of the patients, although in advanced stages, all glutei muscles can be completely atrophic. In general, posterior muscles of the thigh are more severely involved than anterior muscles (A), but is not uncommon to find involvement of vasti muscles, especially vastus intermedius (VI). Atrophy of gastrocnemius medialis (GMed) and soleus (Sol) is frequently found (A), but in advanced cases (B) is not uncommon to find atrophy of gastrocnemius lateralis (GLat), peroneus (Per) and tibialis anterior (TA) muscles.

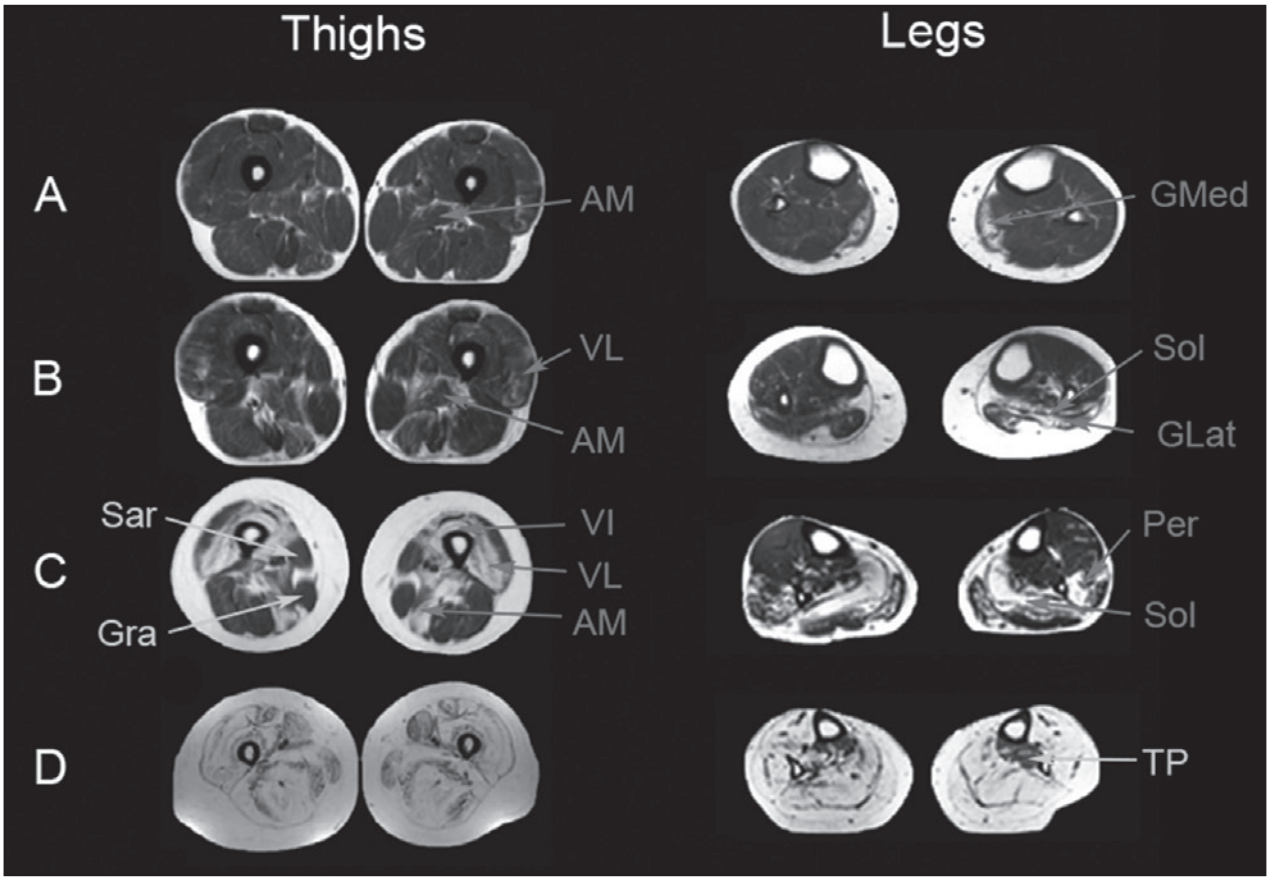

Figure 7. Progression of muscle atrophy in patients with mutations in the DYSF gene. There is a mild involvement of the adductor major (AM) and gastrocnemius medialis (GMed) in the first stages of the disease (A). In a second stage (B), atrophy involves also the vastus lateralis (VL) and the soleus (Sol). Then (C), fatty infiltration progresses and involves the rest of the vasti muscles and the posterior muscles of the thighs, but sartorius (sar) and gracillis (gra) are commonly not involved. In the legs, the vastus lateralis and peroneus become atrophic. In the most advanced stages (D), all the muscles are infiltrated by fat, but in some patients tibialis posterior (TP) may be not involved.

Tibialis posterior is not commonly involved until very late stages of the disease.

Keys for the diagnosis

a. Predominant involvement of posterior muscles of the thighs associated to atrophy of *gastrocnemius medialis* and *soleus*.
b. The lateral part of the *vastus laterallis* is not involved until late stages of the disease. Different to other diseases, *rectus femoris* is also involved in many patients.

2. LGMD2B or disferlinopathy

The reports published on the radiological features of patients with mutation in the DYSF gene have mainly been focused in the study of the lower limbs (Fig. 7). The two most common phenotypes associated to mutations in the DYSF gene, the limb girdle muscle dystrophy and the distal posterior myopathy (Miyoshi myopathy) are not differentiable using MRI (2, 25). Common muscles involved are:

a. Pelvis: *Glutei minimus* is commonly inlvoved in dysferlin patients. However, the *glutei medius* and *maximus* are also involved later on the progression of the disease. Psoas muscles may be not involved until late stages of the disease.
b. Thighs: Adductor muscles are involved from the onset of the disease. The first change is an hyperintense signal in STIR sequences, reflecting the presence of oedema before fatty muscle infiltration is observed in T1 sequences (2). *Vastus lateralis*, *medialis* and *intermedius* are involved early in the progression of the disease. Later on, posterior muscles of the thighs, especially the *semitendinosus* and the *semimembranosus* become atrophic. *Biceps* short head is less affected in many patients, until late stages of the disease when all muscles of the thighs, except *sartorius* and *gracillis* become atrophic.
c. Legs: An hyperintense signal in STIR sequences involving the *gastrocnemius mediallis* is commonly ob-

served at the onset of the diseases. Atrophy involves this muscle first and then progress affecting *gastrocnemius lateralis* and *soleus*. In a more advanced stage antero-lateral muscles are also atrophied. *Tibialis posterior* is not involved until late stages.

Clues for the diagnosis

STIR hyperintensities in adductor and gastrocnemius medialis muscles at the onset of the disease (26, 27).

Involvement of the vasti muscles early in the progression of the disease associated to atrophy of adductor muscles and *gastrocnemius medialis*.

3. Sarcoglycanopathies (LGMD 2C-F)

The radiologic characteristics of the patients with mutations in the sarcoglycan genes are not fully established. There are only some case reports published (24). In our experience, the pattern seems to be homogeneous among patients *(Tasca et al, in preparation).* There is a severe involvement of all *glutei* muscles and the *psoas* in most of the patients reported. In the legs, muscles of the anterior and posterior compartment are also severely affected from the onset without involvement of *sartorius* and *gracillis*. Different to other muscle dystrophies, muscles of the legs are not involved until late stages of the disease (Figure 8).

4. LGMD-2I

LGMD-2I is produced by mutations in the *FKRP* gene. Muscle MRI features have been widely reported (24, 28), and the pattern seems to be very similar to patients with mutation in the *CAPN3* gene.

a. Pelvis: *Glutei* muscles are involved from the onset of the disease, being severely involved in patients in a intermediate stage of the disease. *Psoas* and *illiacus* muscles are not involved until late stages of the disease.

b. Thighs: The posterior compartment of the legs is involved from the onset of the disease. *Biceps*, *semitendinosus* and *semimembranosus* are the most severely involved muscles in these patients. *Vasti* muscles are progressively involved in LGMD-2I patients. *Vastus intermedius* is the most common vasti muscle involved, associated to a variable degree of involvement of *vastus lateralis* and *medialis*. *Rectus femoris* can be involved in many cases, associate to atrophy of the adductor muscle. In later stages all muscles can be involved, being *sartorius* and *gracillis* the less atrophic muscles.

c. Legs: Both *gastrocnemius* and *soleus* are involved from the onset of the disease. In later stages, *peroneus* and *tibialis anterior* become also involved, although they are commonly less atrophic than the posterior muscles of the legs.

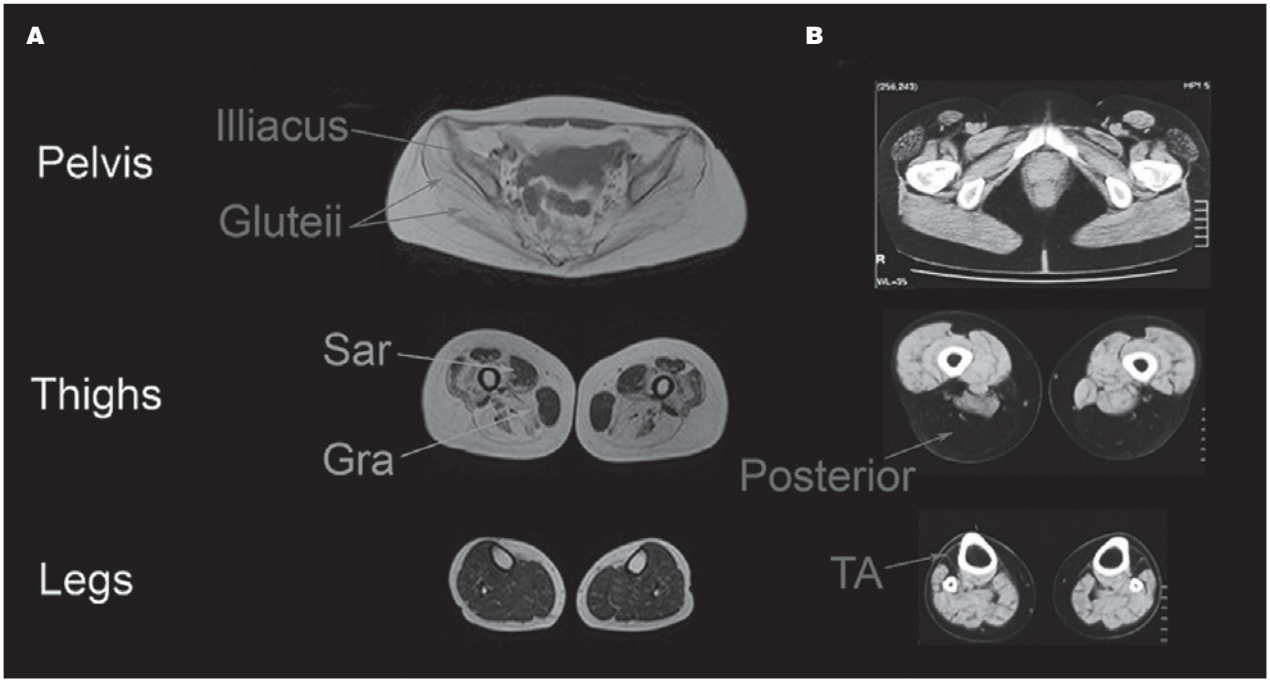

Figure 8. Muscle MRI of patients with sarcoglycanopathy and titinopathy. A: 35 years old patient with LGMD-2D: there is a severe involvement of pelvic muscles (including glutei and psoas muscles) and thigh muscles. Sartorius (sar) and gracillis (gra) are not involved until advanced stages of the diseases. In contrast leg muscles are not involved. B: Muscle CT of a 62 years old patient with distal titinopathy: preferential involvement of the tibialis anterior (TA) and the posterior muscles of the thighs is observed. In contrast, the pelvis muscles are not involved.

Clues for the diagnosis

Severe involvement of glutei muscles associated to atrophy of the posterior muscles of the thighs and legs.

Vastus intermedius is more involved than *lateralis* and *medialis* in many cases. In general atrophy affects first *vastus medialis* than *lateralis*.

5. LGMD2J or Titinopathy

Patients with the classical titin phenotype in which there is a preferential involvement of the *tibialis anterior*, have a very characteristic muscle MRI (7). It is very common to find an isolated atrophy of the *tibialis anterior* muscle, that can be the only muscle affected during many years. Later on the progression of the disease, atrophy can also affect *soleus* muscles, the posterior compartment of the thighs and the *rectus femoris* (Fig. 8).

In recent years, many different phenotypes of muscle disease have been associated to mutations in the *TTN* gene, including congenital myopathies or patients with proximal weakness of the lower and upper extremities. There is not a clear muscle MRI pattern in these cases.

However, patients with the hereditary myopathy and early respiratory failure phenotype (HMERF), have a common pattern of muscle MRI involvement. It has been reported that *illiacus*, *psoas*, *obturator*, *semitendinosus*, *sartorius* and *gracillis* are commonly involved in these patients.

6. LGMD2L

LGMD 2L is produced by mutations in the *ANO5* gene. Although this disease has been recently described, the muscle MRI pattern is very well known and seems to be homogenous among patients (29, 30) (Fig. 9). In these cases, muscle MRI features are very similar to those de-

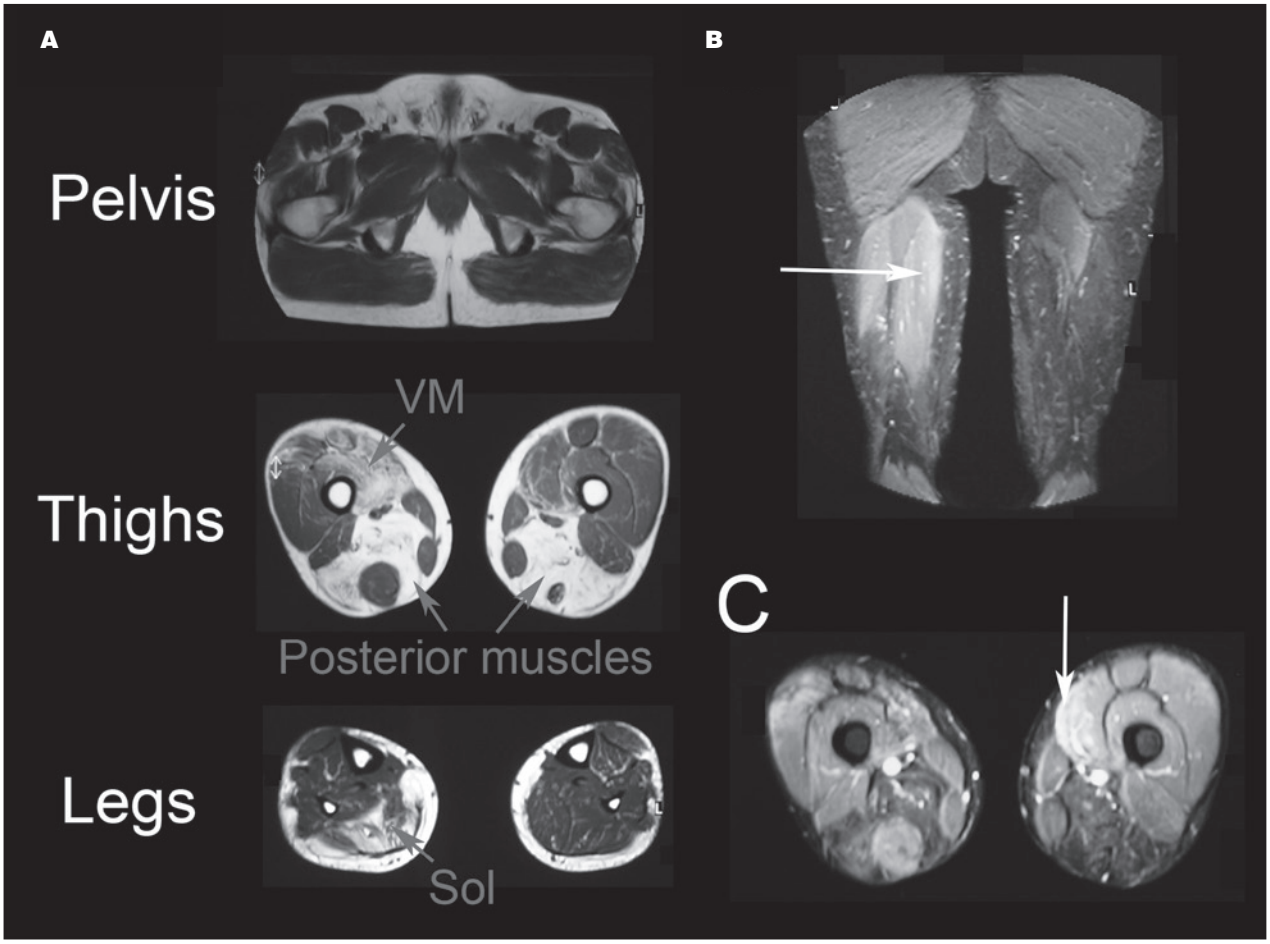

Figure 9. Muscle MRI of a patient with mutations in the ANO5 gene. A: Muscle MRI of a 53 years old patient with mild involvement of the lower limbs. Axial images showed no involvement of pelvic muscles, but in contrast there was a clear asymmetric involvement of vastus lateralis (VL) and posterior muscles of the thighs. In the legs, muscles of the posterior compartment were also involved, including soleus (Sol) and gastrocnemius medialis. B and C: hypenintensities in STIR sequences were observed in the posterior muscles of the thighs and in the vastus medialis muscle (arrows).

scribed in patients with mutations in the DYSF gene (31). The common muscles involved are:

a. Pelvis: *Glutei*, *psoas* and *illiacus* muscles are commonly not involved until late stages of the disease.

b. Thighs: *Adductor* muscles and posterior muscles of the thighs, especially the *semitendinosus* (32), are involved from the onset of the disease. As the weakness progresses there is an involvement of the *vastus lateralis, medialis* and *intermedius*. As in dysferlinopathy patients, *sartorius, gracilllis* and *rectus femoris* are not involved until late stages. STIR hyperintensisities are commonly observed in many muscles before atrophy is clearly detected in T1 sequences. The presence of asymmetries in the degree of muscle atrophy is very common among patients.

c. Legs: *Soleus* and *gastrocnemius medialis* are commonly involved from the onset of the disease, followed by atrophy of the *gastrocnemius lateralis*, *peroneus* and *tibialis anterior* muscles.

Clues for the diagnosis

Asymmetric involvement of the thighs muscles, especially in the degree of involvement of the *vasti* muscles.

Glutei muscles are less involved than muscles of the thighs and legs until very late in the disease.

Increase in the STIR signal is observed in many cases from the onset of the disease.

Muscle MRI in other hereditary myopathies

1. Adult onset Pompe disease

Pompe disease, also known as glycogenosis type II, can manifest as slowly progressive weakness involving axial and proximal muscles of the limbs. Pompe patients are in many cases misdiagnosed as patients with muscle dystrophies. Muscle MRI is very useful to identify these patients, as the pattern has been widely described and seems to be very characteristic (Fig. 10). There is a preferential involvement of tongue from the onset of the disease, also in patients without clear clinical involvement of this muscle. Periscapular muscles are commonly involved, especially the *subscapularis* muscle that is atrophic in many cases (33). Paravertebral muscles are affected early in the progression of the disease, especially the *multifidus* and *longissimus* muscle (34). Atrophy of the abdominal muscles runs parallel to progression of the disease. Patients may develop a severe atrophy of *rectus abdominalis*, *obliquus internus* and *externus* and *trasversus abdominalis*. Glutei muscles are also involved in all patients. In general these muscles are more involved than *psoas-illiacus* muscles. The involvement of the adductor muscles occurs early in the progression of the disease followed by involvement of posterior muscles of the thighs, *vastus intermedius* and finally *vastus lateralis* and *medialis* (35). In most of the cases, there is not involvement of the muscles of the legs, than can be normal also in wheel-chair bound patients.

2. Myopathies associated to mutations in the VCP gene

Mutations in the VCP gene have been associated to a characteristic triad of diseases: myopathy with proximal and/or distal involvement in the upper and lower limbs, Paget disease and fronto-temporal dementia (36). Although the muscle MRI pattern has not been published, in our experience atrophy involves muscles of the pelvis, thighs and legs. The most characteristic finding in this disease is that fatty infiltration is not homogeneous and in many cases shows a patchy distribution among the muscles (Fig. 11).

3. Oculo-pharingeal muscle dystrophy

OPMD disease is characterized by the triad of ptosis, dysphagia and proximal weakness of the lower and upper limbs. Although the symptoms are very suggestive, sometimes it can be difficult to differentiate these patients from patients with mitochondrial myopathy. In our experience, muscle MRI is useful in this case. Muscle MRI of the pelvis shows a progressive involvement of glutei muscles, specially *glutei minimus*. Psoas is also involved early in the progression of the disease. In the thighs there is a preferential involvement of posterior muscles of the thighs and of the adductor muscles. In the legs, s*oleus and peroneus muscles* are commonly involved early. In fact, soleus is commonly more atrophic than gastrocnemius muscles also in advanced patients (37) (Fig. 11).

4. Collagen VI related myopathies.

Although mutations in one of the three COLVI genes have been commonly related with congenital muscle dystrophies, this disease can also present during the adulthood. Muscle MRI is highly specific and has been described elsewhere. The main finding is a muscle atrophy involving the periphery of the *vastus lateralis* and *medialis*, while the center of the muscle is not involved (38). This pattern has been described as *concentric atrophy* (Fig. 11). A similar pattern can also be observed in the *gastrocnemius medialis*, where there is a ring of atrophy between the *gastrocnemius medialis* and *soleus* muscle. Muscle atrophy can also involve other muscles, such as the *rectus femoris* muscle, the posterior compartment of the thighs, the paravertebral muscles and the upper limbs muscles. In this latter case, a band of muscle atrophy is commonly observed in the *triceps* and in the *subscapu-*

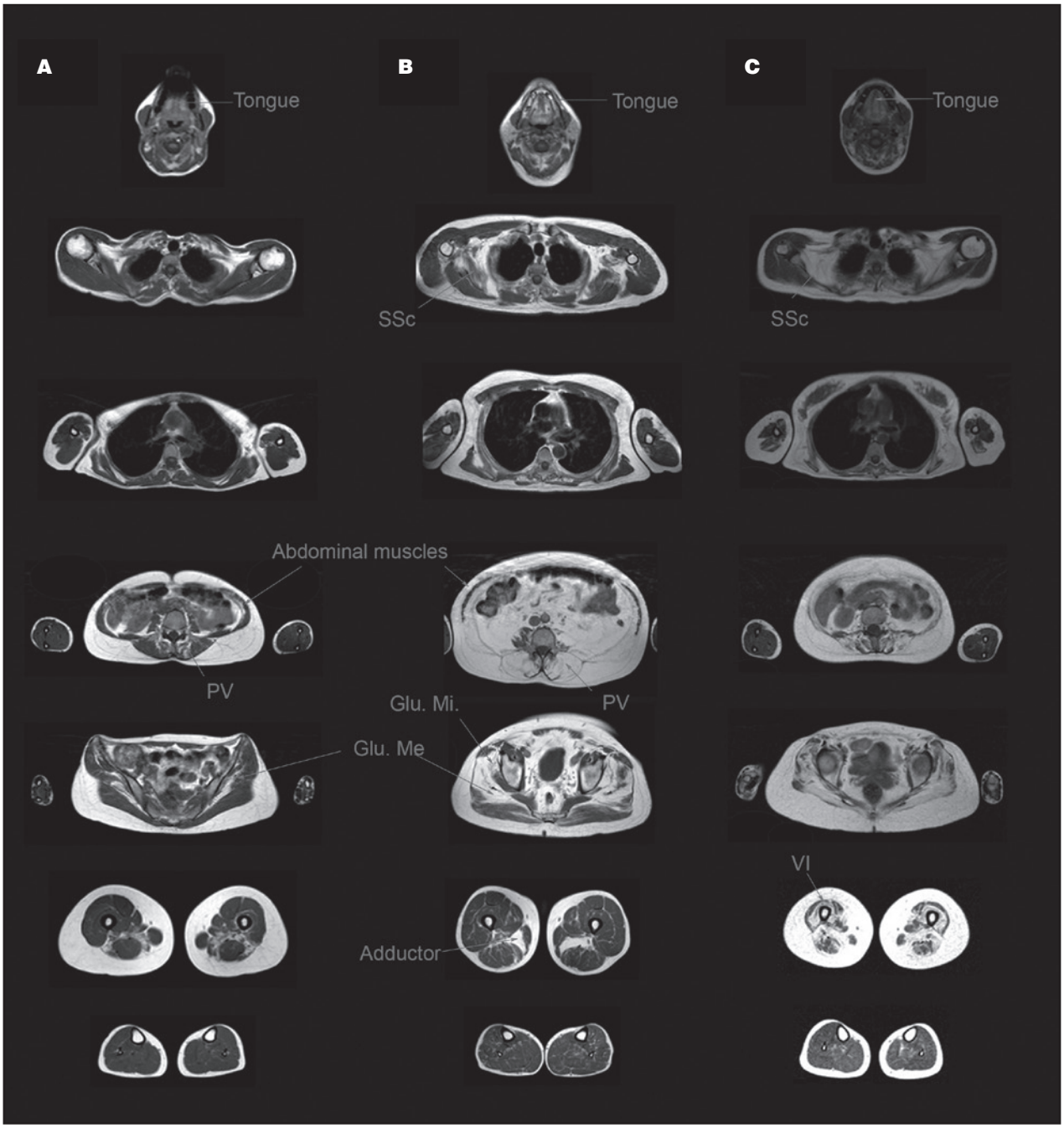

Figure 10. Muscle MRI of patients with Pompe disease. A: Whole body muscle MRI of a 35 years olf patient with mild muscle involvement showing atrophy of tongue, paravertebral and abdominal muscles, gluteus medius (Glu.Me) and posterior muscles of the thighs. B: Muscle MRI of a 43 years old patients with moderate weakness showing involvement of tongue, subscapularis (SSc), paravertebral and abdominal muscles, gluteus medius (Glu.Me) and gluteus minimus and adductor major (AM). C: Muscle MRI of a 44 years old patients with severe weakness showing involvement of tongue, subscapularis (SSc), paravertebral, abdominal, all glutei muscles and anterior and posterior muscles of the thighs.

laris. It has been suggested than in those patients with unknown mutations showing this characteristic pattern, genetic tests can be performed without histologic demonstration of collagen VI deficiency.

5. Facio-scapulo-humeral muscle dystrophy (FSHD)

The clinical diagnosis of FSHD is easy to achieve based only in clinical symptoms in most of the patients. However, muscle MRI studies can be helpful in un-

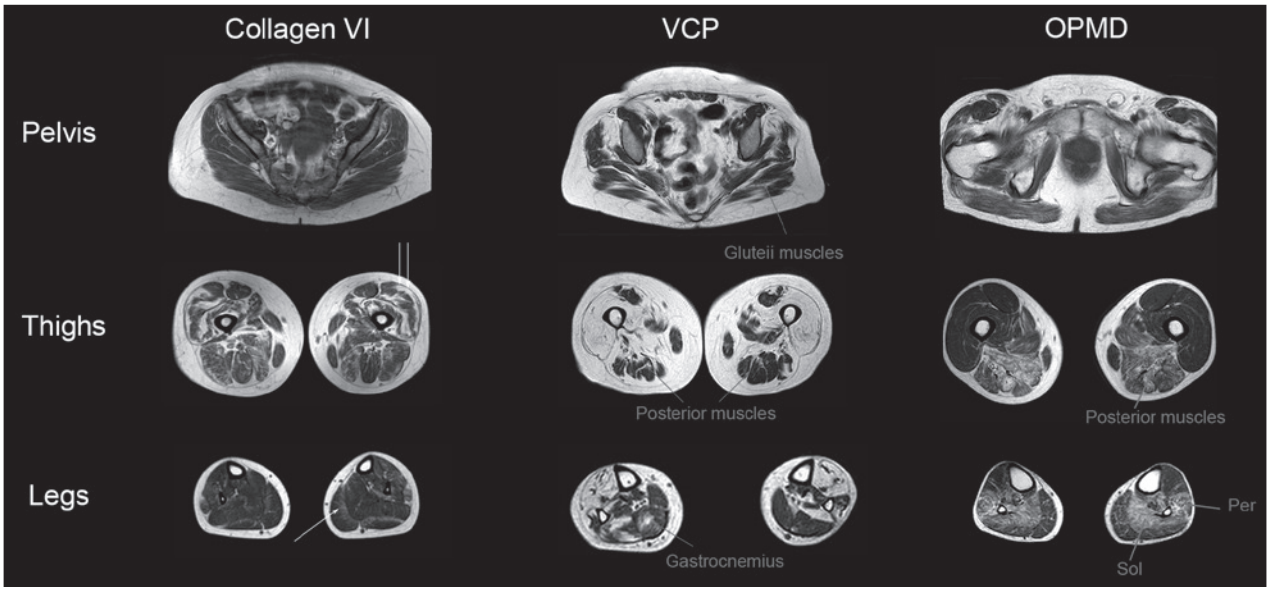

Figure 11. Muscle MRI of patients with collagen VI related myopathy, HIBMPD and OPMD muscle dystrophies. A: Muscle MRI of a 20 years old patient with mutations in the COL6A3 gene showing concentric atrophy of the vastus lateralis (double arrow) and a band of atrophy between gastrocnemius medialis and soleus (single arrow). B: Muscle MRI of a 56 years old woman with a mutation in the VCP gene producing patchy atrophy of the glutei, posterior muscles of the thighs and both gastrocnemius. C: Muscle MRI of a 72 years old patient with OPMD showing preferential involvement of posterior muscles of the thighs and the soleus muscle.

clear cases. Although FSHD is one of the most common muscle diseases, there are not enough studies on which patterns are commonly observed in this disease. Tasca and collaborators studied MRI muscle involvement of the upper limbs and reported a preferential involvement of periscapular muscles including *trapezius*, *serratus anterior*, *latissimus dorsi, pectoralis major* and *rhomboids* (39). In contrast, *supraspinatus*, *infraspinatus* and *subscapularis* were not commonly involved in this disease. The frequency of asymmetric involvement was very high. The studies focused on lower limbs have shown a predominant involvement of *Tibialis anterior* in most of the cases (40). Other muscles can also be atrophic such as the vastii, the posterior compartment of the thighs and both *gastrocnemius*. Asymmetric changes were also very common in the lower limbs, and this is probably the most important clue for the radiologic diagnosis of the disease (Fig. 12).

Conclusions

Muscle MRI has become a useful tool in the diagnosis of patients with muscle dystrophies. The standardization of the study protocols has allowed obtaining information from many patients with different muscle diseases. But still, most of the studies published are performed in relatively small cohorts of patients and are in general transversal. More information regarding progression of the atrophy during the natural history of the disease is needed. Collaboration between different study groups is necessary to increase the number of patients analyzed and to include patients in different clinical stages (from presymptomatic to advanced patients). In this sense the effort performed by the European Comission creating an E-COST action centered in the application of muscle MRI to the study of muscle diseases is remarkable (http://myomri.eu). Patients associations have also shown interest in radiological studies. This is the case of the Jain Foundation that sponsors a clinical-radiological study to describe the natural history of patients with LGMD-2B that has already recruited 196 patients all over the world (http://www.jain-foundation.org). Although the diagnosis of muscle dystrophies still continues to be based on a careful clinical history, a detailed physical examination and a complete study of the muscle biopsy, information on the muscle MRI pattern could be helpful for the genetic diagnosis of these diseases. Along the same lines, a high level of expertise is needed to identify muscle MRI patterns, that in many cases are not patognomonic of a single disease (41). In this sense, the creation of training schools will improve the level of knowledge among radiologists and neurologists all over the world, making muscle MRI studies more useful for clinical practice.

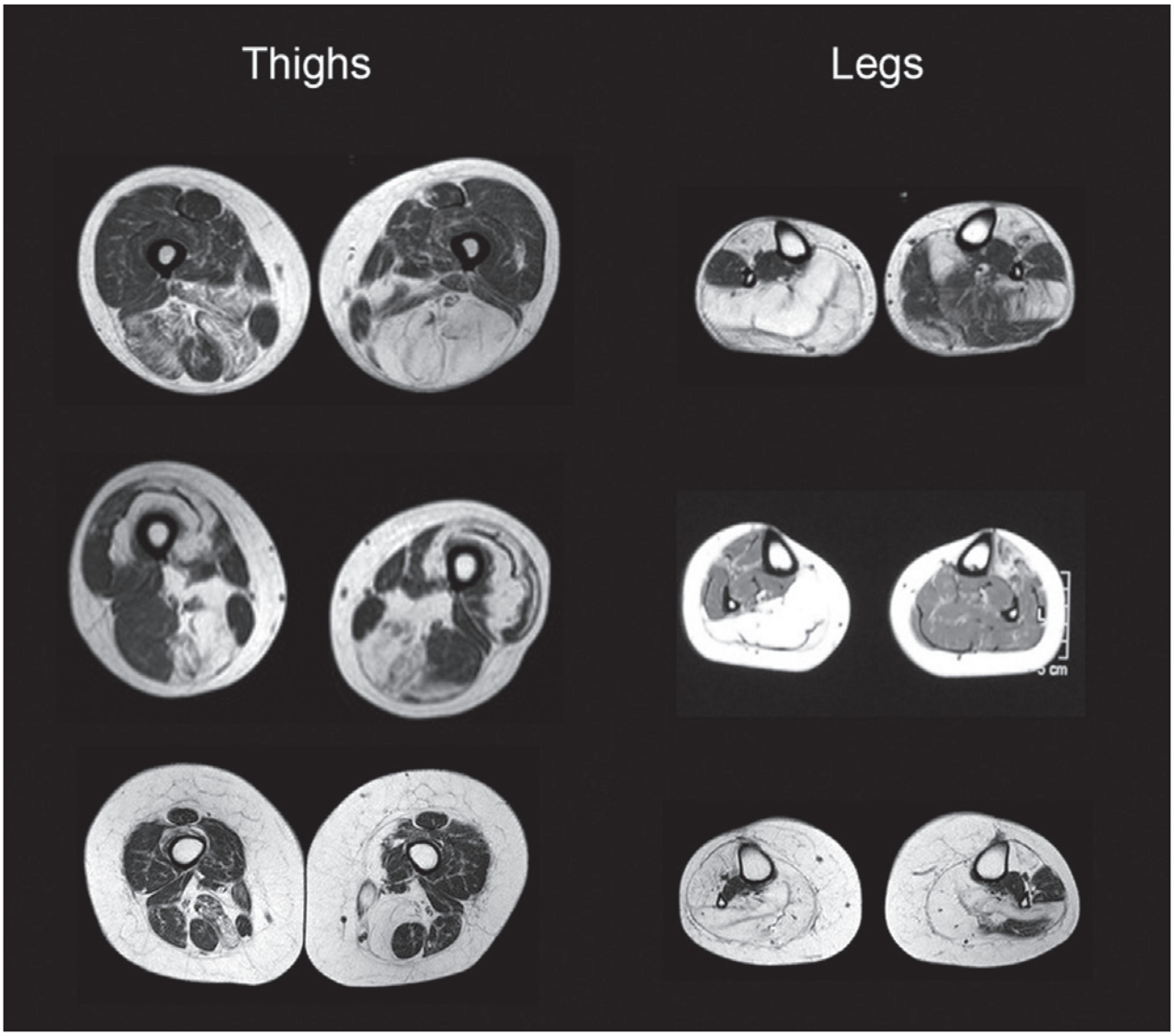

Figure 12. Muscle MRI of patients with Facio-Scapulo-Humeral muscle dystrophy. Muscle MRI of different patients with FSH muscle dystrophy showing asymmetries in the thighs and legs studies.

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Imaging alterations in skeletal muscle channelopathies: a study in 15 patients

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Skeletal muscle channelopathies (SMC), including non dystrophic myotonias (NDM) and periodic paralyses (PP), are characterized by considerable clinical overlap and clinical features not always allow addressing molecular diagnosis. Muscle imaging has been shown to be useful for differential diagnosis in neuromuscular disorders, however it has been relatively poorly investigated in SMC.

We studied 15 patients affected by genetically confirmed SMC (NDM = 9, PP = 6) through muscle MRI or CT of thighs and legs, including 11 patients mutated in *SCN4A* gene, 2 in *CACNA1S* and 2 in *CLCN1*. Mean age at muscle imaging was 45.2 ± 18 years (range 22-70).

Overall, fatty infiltration was found in thigh muscles in 8 (53%) patients and in leg muscles in 10 (60%). All patients mutated in *CLCN1* and *CACNA1S* had abnormal thigh and/or leg muscle MRI, regardless the disease duration. On the contrary normal thigh and leg muscle MRI or CT scans were observed in 4/15 (27%) patients, all mutated in *SCN4A*. Variable degrees of fatty changes were found in patients mutated in *SCN4A, CACNA1S* and *CLCN1*. No differences on overall score of fatty infiltration were detected between NDM and PP (p-value = 0.953) neither between presence or absence of permanent weakness (p-value = 0.951).

Our data confirm the presence of muscle fatty changes in the majority of SMC patients, although without any specific pattern of involvement. However muscle MRI may be a useful tool for longitudinal follow-up of SMC patients, in particular to evaluate the occurrence and the progression of fixed myopathy.

Key words: muscle MRI, periodic paralyses, non dystrophic myotonias, SCN4A, CACNA1S, CLCN1

Introduction

Skeletal muscle channelopathies (SMC) are rare (prevalence in UK: 1.12/100000) neuromuscular disorders clinically characterized by paralytic attacks and/or myotonia (1); on the basis of the predominant clinical feature SMC are classified as non dystrophic myotonias (NDM) or periodic paralyses (PP) (1). NDM include myotonia congenita (MC), paramyotonia congenita (PMC) and sodium channel myotonia (SCM) (1, 2). PP comprise hypokaliemic periodic paralysis (HypoPP), hyperkaliemic periodic paralysis (HyperPP) and Andersen-Tawil syndrome (ATS) (1, 2). SMC are dominantly inherited diseases, except for recessive MC, and are caused by mutations in genes encoding skeletal muscle ion channels that affect muscle excitability. In particular, MC are due to mutations in *CLCN1* gene, encoding the voltage-gated chloride channel (CLC-1), PMC, SCM, HypoPP type II and HyperPP to mutations in *SCN4A*, encoding the alpha subunit of the voltage-gated sodium channel (NaV1.4), HypoPP type I to mutations in *CACNA1S*, encoding the voltage-gated calcium channel (CaV1.1), and ATS to mutations in *KCNJ2*, encoding the inward-rectifying potassium channel (Kir2.1).

Considerable overlap exists among different SMC entities, being myotonia also present in HyperPP and paralytic attacks described even in PMC, hence clinical features not always allow to address molecular diagnosis (2).

In the last decade muscle MRI has been proven to be a highly reliable, non-invasive tool for the differential diagnosis and the evaluation of progression of muscular diseases, revealing specific patterns of muscle involvement associated with different myopathies (3-8). However muscle MRI has been relatively poorly investigated in SMC (9-13).

Aim of our study was to investigate the presence and severity of fatty infiltration in lower limb muscles through MRI or CT scan in a cohort of patients with genetically determined SMC.

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Methods

Subjects

We investigated retrospectively 15 patients (11 males, 4 females) affected by genetically characterized SMC (NDM = 9, PP = 6) and undergone muscle MRI or CT scan in our Institute during the period ranging from January 2008 and December 2013. Eleven patients were mutated in *SCN4A*, 2 in *CLCN1* and 2 in *CACNA1S*. Among patients with mutations in *SCN4A*, 4 had PMC, 2 SCM, 2 HypoPP type II and 2 HyperPP. Mean age at onset was 10.2 ± 8.7 years (range 1-30); mean age at muscle imaging was 45.2 ± 18 years (range 22-70). Mean time from symptom onset to muscle imaging was 36.6 ± 14.9 years (range 8-62).

Muscle imaging protocol

Nine (*SCN4A* = 5, *CLCN1* = 2, *CACNA1S* = 2) out of 15 patients underwent muscle MRI and 6 (*SCN4A* = 6) CT scan. In first subgroup patients were scanned with MRI at 1.5T (Philips Achieva, Eindhoven, Holland) in a supine position with surface array coils. Patients were scanned with a clinical protocol comprising T1w axial and coronal images for the simultaneous bilateral evaluation of leg and thigh (representative parameters: matrix for coronal images 504 x 298 in the thigh, 452 x 262 in the leg, for axial images 448 x 214 both in thigh and leg; number of signal averages (NEX): 1 for coronal, 2 for axial images; leg coronal: repetition time (TR): 572 mms, echo time (TE): 10 mms, thickness: 3 mm, 20 slices; leg axial: TR: 467 mms, TE: 4.4 mms, thickness: 10 mm, 40 slices; thigh coronal: TR: 570 mms, TE: 10 mms, thickness: 4 mm, 30 slices; thigh axial: TR: 467 mms, TE: 4.4 mms, thickness: 10 mm, 40 slices) with 1 mm slice gap.

CT scan (Philips Brilliance™ CT, Eindhoven, Holland) comprised axial images (representative parameters: 512 x 512 matrix, 120 kV, 400mAs, FOV-field of view-500) with 2 slides for thigh and leg, respectively, with 4.5 mm thickness and 2 cm slice gap. Scanning time was about 20 minutes for MRI and 5 minutes for CT.

Muscle imaging analysis

Lower limb muscles were analyzed for fatty infiltration and then the overall degree of involvement at thigh and leg level was categorized. The following muscles were assessed on both sides: rectus femoris, vastus lateralis, vastus intermedius, vastus medialis, adductor magnus, gracilis, sartorius, biceps femoris, semimembranosus and semitendinosus in the thigh; tibialis anterior, peroneus longus, tibialis posterior, soleus, medial and gastrocnemius in the leg. These 32 muscles were assessed on T1w sequences for the presence of fatty infiltration using Fischer's semi-quantitative scale (5): 0 – normal appearance, 1 – occasional scattered T1 hyperintensity, 2 – confluent areas of T1 hyperintensity < 50% of muscle involved, 3 – confluent areas of T1 hyperintensity > 50%, 4 – complete replacement of muscle with fat. Finally muscle involvement of thigh and leg, respectively, was categorized as already reported by Morrow and colleagues (12):

- Normal: all muscles grade 0.
- Mild limited changes: grade 1 changes in £50% of the muscles (10/20 in thighs; 6/12 in legs).
- Mild extensive changes: grade 1 changes in > 50% of the muscles.
- Marked changes: any muscle with grade 2 changes.

The same analysis, although initially set up for MRI images, was performed also for muscle CT scan.

Statistical analysis

Overall score was defined as the mean fatty infiltration detected by muscle imaging for all investigated muscles in each patient. Spearman's rank correlation coefficient was used to assess correlation between overall score and age. Differences on the overall score between independent groups (phenotypes and presence/absence of permanent weakness) were investigated with Mann-Whitney test. P-value less than 0.05 was considered as significant. Statistical analyses were performed using SPSS 20.0.

Results

Overall, normal thigh and leg muscle MRI or CT scans were observed in 4 out of 15 (26.7%) patients, all mutated in *SCN4A* (PMC = 2, SCM = 1, HypoPP type II = 1); among them 1 had muscle MRI and 3 only CT scan. All patients mutated in *CLCN1* and *CACNA1S* had abnormal thigh and/or leg muscle MRI, regardless the disease duration. Patients clinical, genetic and muscle imaging data are shown in Table 1.

Fatty infiltration was found in thigh muscles in 8 (53.3%) patients and in leg muscles in 10 (60%). In particular abnormal findings were observed in 94 out of 300 (31.3%) evaluated thigh muscles and in 65 out of 180 (36.1%) leg muscles. Of note, none of the investigated muscles resulted normal in all patients. Muscles displaying more commonly fatty infiltration were soleus (60%) and medial gastrocnemius (50%); on the other hand muscles more commonly showing no fatty changes were tibialis anterior and rectus femoris (86.7%). Distribution and severity of fatty infiltration in individual thigh and leg muscles of investigated patients are shown in Figure 1.

Patients with abnormal muscle imaging had variable degree of fatty infiltration. In the thigh we observed grade 1 in 55 out of 300 muscles (8 patients), grade 2 in 21

Table 1. Patients clinical, genetic and muscle imaging data.

Pt, Sex	Gene	Mutation	Phenotype	Age at onset (y)	Paralysis	Myotonia	Fixed weakness	Treatment	Age at MRI/CT (y)	Thigh imaging	Leg imaging	Overall score
1,F	SCN4A	R1448C	PMC	5.5	yes	C,H,LL	no	Mex	40 (MRI)	-	-	0.00
2,M	SCN4A	T1313M	PMC	17	yes	C,H	no	Mex	48 (MRI)	+	±	0.56
3,M	SCN4A	T1313M	PMC	2	yes	C,H,LL	A,P,D,F	Mex	64 (MRI)	++	++	0.81
4,F	SCN4A	T1313M	PMC	2	yes	C,H,LL	F	Mex	33 (MRI)	±	±	0.16
5,M	SCN4A	R1448C	PMC	1.5	yes	C,H,LL	P,D	none	30 (CT)	-	-	0
6,F	SCN4A	N275K	SCM	13.5	no	C,LL	A,P	none	63 (CT)	-	-	0
7,M	SCN4A	V445L	SCM	17	no	C,LL	P	Mex	68 (CT)	-	++	0.31
8,M	SCN4A	R675G	HyperPP	1	yes	no	P,D	Acz	45 (CT)	++	++	1.87
9,M	SCN4A	I692M	HyperPP	6	yes	C,H,LL	A,P,D	Hyct	53 (MRI)	++	++	0.94
10,F	SCN4A	T704M	HypoPP II	5	yes	no	no	Hyct	22 (CT)	±	-	0.19
11,M	SCN4A	R672C	HypoPP II	14	yes	no	no	Acz	30 (CT)	-	-	0
12,M	CLCN1	homo R496S	Becker	3	no	C,H,LL	P,D	none	50 (MRI)	++	++	1.25
13,M	CLCN1	c.IVS13+5-11delGTTCTGA + F167L	Thomsen*	30	no	H,LL	P,D	none	70 (MRI)	-	+	0.06
14,M	CACNA1S	R528H	HypoPP I	20	yes	no	A,P,D	No	63 (MRI)	++	++	2.40
15,M	CACNA1S	R528H	HypoPP I	16	yes	no	no	Acz	24 (MRI)	-	+	0.31

Pt: patient; F: female; M: male; homo: homozygous; PMC: paramyotonia congenita; SCM: sodium channel myotonia; HyperPP: hyperkaliemic periodic paralysis; HypoPP: hypokaliemic periodic paralysis; y: years; C: cranial; H: handgrip; LL: lower limb; A: axial: P: proximal; D:distal; F: facial; Mex: mexiletine; Acz: acetazolamide; Hyct: hydrochlorothiazide; -: all muscles normal; ±: mild limited changes; +: mild extensive changes; ++: marked changes (as defined in the methods section).

* Patient 13 was clinically considered as Thomsen myotonia, despite the 2 recessive mutations, due to relative mild phenotype. Compound muscle action potential after short exercise test with and without cooling revealed a pattern compatible with dominant myotonia congenita.

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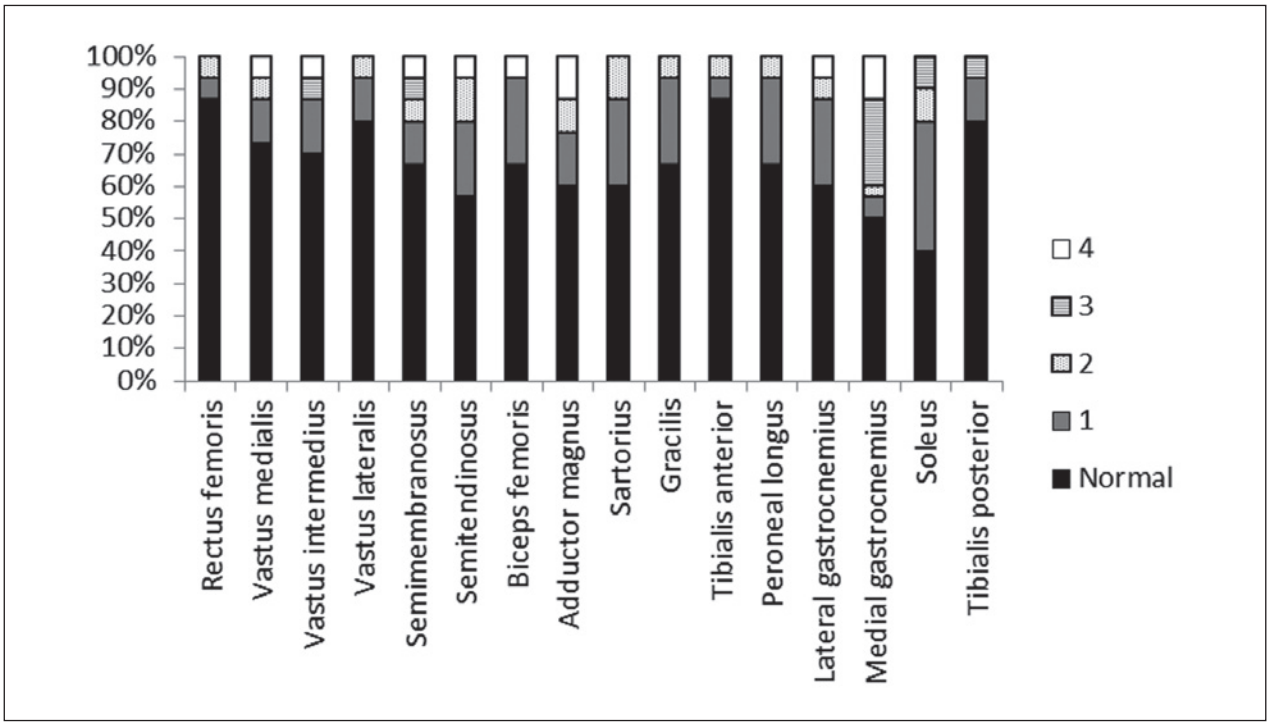

Figure 1. Distribution and severity of fatty infiltration in thigh and leg muscles of the investigated patients. Severity of fatty infiltration was categorized for each muscle using Fischer's semi-quantitative scale as described in the methods section.

muscles (5 patients), grade 3 in 4 muscles (2 patients) and grade 4 in 14 muscles (2 patients); in the leg: grade 1 in 36 out of 180 muscles (7 patients), grade 2 in 10 muscles (3 patients), grade 3 in 13 muscles (5 patients) and grade 4 in 6 muscles (2 patients). Grade 4 was found in thigh muscles in 2 patients and in leg muscles in 2 other patients and muscles more frequently affected were adductor magnus and medial gastrocnemius. Interestingly, patients displaying grade 4 fatty infiltration in thigh had also a consistent involvement (grade 2-3) in leg muscles, contrary to patients with grade 4 in leg muscles in which fatty changes were limited to the calves. Fatty infiltration was almost invariably symmetrical; among 240 muscles investigated on both sides in thighs and legs only 7 (2.9%) muscles from 6 different patients showed asymmetrical involvement, usually differing for 1-grade severity in Fischer's semiquantitative scale, mostly with right side worse than the left.

No specific pattern of muscle involvement was observed in different clinical phenotypes which were associated with variable degrees of fatty changes, as revealed by overall score (see Table 1 for details and figure 2 for images from patients); this value was higher in both 2 patients affected by HyperPP, in 1 patient with recessive MD and in 1 with HypoPP type I. To this purpose the highest value of overall score was reached in patient 14 with HypoPP type I, showing severe progressive myopathy since the age of 54, after 2 years from the cessation of paralytic attacks. His son (patient 15) had only initial minimal changes at muscle MRI performed at the age of 24, without any detectable muscle weakness at neurological examination, although with relatively frequent paralytic attacks.

Fixed muscle weakness was evident at neurological examination in 10 out of 15 (66.7%) patients (*SCN4A* = 7, *CLCN1* = 2 and *CACNA1S* = 1), mainly in proximal lower limbs (9/10). All these 10 patients had variable degree of muscle involvement at muscle imaging, except for patient 5 and 6 (1 PMC and 1 SCM), showing normal lower limb CT scan.

No Spearman's rank correlation was found between overall score and age at muscle imaging ($r = 0.288$, p-value = 0.298) or disease duration ($r = 0.394$, p-value = 0.146). No differences on overall score between NDM and PP (p-value = 0.953) neither between presence or absence of fixed weakness (p-value = 0.951) were found.

Discussion

In recent years muscle imaging has been widely used to support clinical diagnosis of neuromuscular disorders

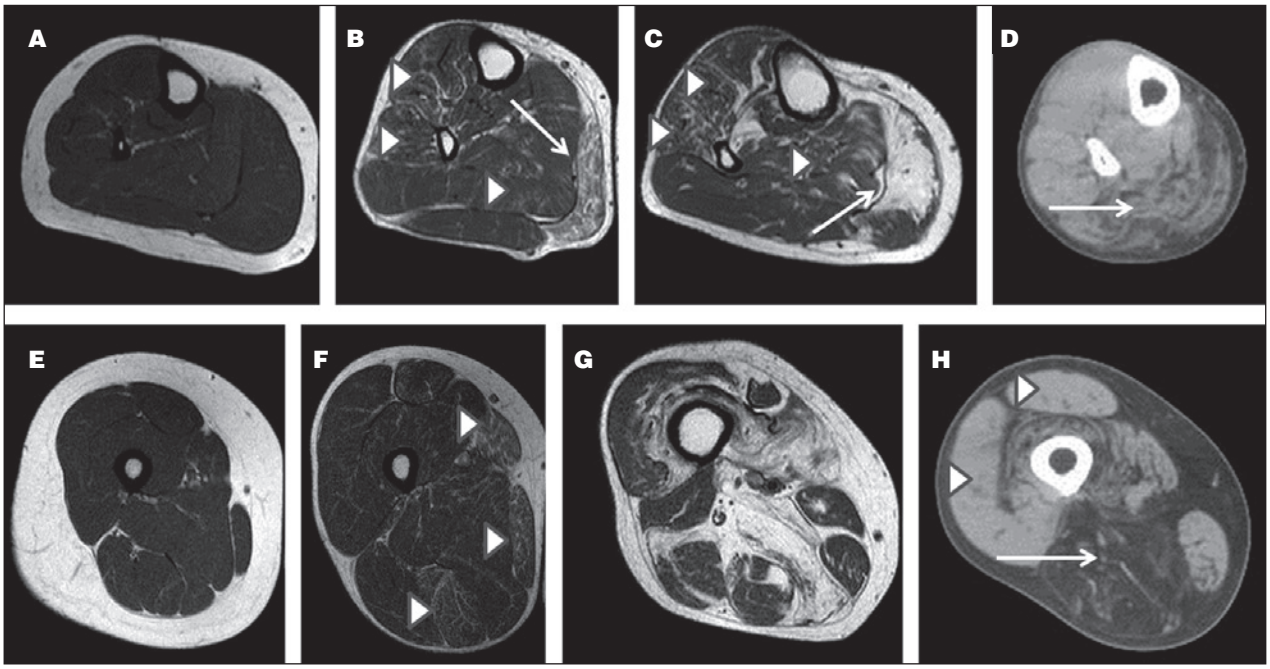

Figure 2. Normal leg (A) and thigh (E) muscle MRI in a patient affected by PMC. Marked T1w changes in medial gastrocnemius (arrows) in a patient with recessive MC (B) and in a patient with HypoPP type I (C) in association with lower severity involvement of soleus, peroneal longus and tibial anterior (arrowheads). Both patients had also thigh involvement, limited to sartorius, gracilis and semitendinosus (arrowheads) in recessive MC (F), more marked and diffuse in HypoPP type I (G). Muscle CT scan hypodensity (arrows) limited to posterior leg (D) and predominant in posterior thigh (H) in a patient with HyperPP. To be noticed the sparing of rectus and vastus lateralis (arrowheads).

and for their differential diagnosis through the identification of specific patterns of muscle involvement. However, few data are available in literature on muscle imaging in SMC (9-13).

Here, we report our experience in a cohort of patients affected by SMC investigated with muscle MRI or CT scan to evaluate the frequency and severity of muscle fatty infiltration. A recent study including a slightly wider cohort of patients was focused only on NDM (12); on the contrary in our study we investigated also PP due to *SCN4A* and *CACNA1S* gene mutations.

Our findings revealed that fatty infiltration was found in thigh muscles in about 50% of the patients and in leg muscles in about 60%. In particular abnormal findings were observed in about one third of evaluated thigh and leg muscles, in agreement with the aforementioned study (12). On the contrary, a study investigating 3 patients with recessive MC through whole body MRI did not demonstrate any abnormality (10).

Grade 1 fatty infiltration was detected in more than a half of abnormal muscles, supporting the hypothesis that severe fatty infiltration is not a predominant finding in SMC. Unfortunately, our study did not include comparative data in age-matched normal individuals, making difficult to evaluate the pathological meaning of the mild changes detected by muscle imaging in our patients. However the study by Morrow and colleagues revealed mild limited T1w changes also in healthy volunteers, in particular in leg muscles (12); hence the slight and limited muscle fatty infiltration should be considered as a negligible and unspecific finding. Higher severity of fatty infiltration (grade 3 and 4) were slightly more frequent in our cohort than in the aforementioned study (12), probably because we included also patients affected by PP, which may be associated with the development of progressive myopathy (14). Of note, higher severity of fatty infiltration was observed more frequently in medial gastrocnemius, although this should be considered an unspecific finding, being reported also in other myopathies (15).

Within the limitation of a small cohort of patients, we did not confirm the correlation between age at muscle imaging and overall score detected by Morrow and colleagues (12), probably due to the lower rate of patients with recessive MC, for which the correlation was stronger; moreover we did not find any correlation between disease duration and overall score.

Contrary to other muscle diseases (3-8), no specific pattern of muscle involvement was observed, in agreement with Morrow and colleagues (12). Overall score was very variable among different phenotypes and no

significant differences were found between NDM and PP. However, among PP patients overall score was higher in both HyperPP cases, which had also fixed weakness, than in those affected by HypoPP type II, without any detectable weakness. Considering also that the highest overall score was observed in a patient mutated in *CACNA1S,* our data support the hypothesis that patients affected by HypoPP type I or HyperPP develop more frequently a progressive myopathy than those with HypoPP type II (14). Of note, fatty infiltration in PP has been investigated previously only in one patient affected by HypoPP type I, revealing diffuse degeneration of calf muscles, except the tibialis anterior (16); no data on thigh muscle were provided. Among 9 NDM patients we detected fatty infiltration in thigh and leg muscles about in one and two third of the patients, respectively, similarly to findings already reported (12).

Our study had some limitations. First, MRI STIR sequences have not been performed; STIR hyperintensity reflecting oedema has been found in patients with NDM, in particular in medial gastrocnemius ("central stripe") (12); the central stripe has not been reported in healthy volunteers or in other conditions, suggesting a possible specific feature of NDM. Second, we used a 1.5T and not 3T MRI as in the aforementioned stud (12). Third, we included 6 patients with muscle CT scan, which is less sensitive than MRI, in particular for the detection of oedema (15), hence some subtle changes in the muscle might be missed. Despite the last two limitations, our results in terms of detection of fatty infiltration are similar to those reported by Morrow and colleagues (12).

In recent years innovative muscle imaging approaches have been carried out in SMC. 3T muscle MRI showed muscle oedema in HypoPP type I and II similarly to healthy subjects after exercise, although with higher severity and more frequent involvement of the calf muscles (13). Of note, HypoPP patients were investigated during the interval period between the episodes of paralysis, suggesting the presence of muscle oedema also when the patient was asymptomatic. Thus muscle MRI may be useful for monitoring treatment effects in between paralytic attacks. Similarly, 3-T sodium ^{23}Na MRI has been studied in HyperPP revealing Na^+ accumulation during weakness episodes after provocation with specific triggers and showed increased myoplasmic Na+ content in HyperPP patients with permanent weakness compared to those with only episodic weakness (9, 11); this suggests that Na+ overload may cause muscle degeneration developing with age, thus ^{23}Na MRI may evaluate the possible efficacy of treatments that reduces this overload. In this regard remarkable benefit of acetazolamide on permanent weakness in a patient affected by HyperPP has been documented clinically and through MRI, which revealed a significant increase in muscle bulk (17). Sodium accumulation has been also documented by ^{23}Na MRI in a HypoPP patient mutated in *CACNA1S* (16). A further study focused on in vivo imaging of chloride and sodium in patients with Hypo PP type I through ^{35}Cl and ^{23}NA MRI, showing increased muscle concentrations of both sodium and chloride compared to healthy volunteers (18). At last, orbital MRI revealed extraocular muscle hypertrophy in 2 NDM patients mutated in *SCN4A* (19) At present most of the imaging techniques are limited to research context and further studies are needed to clarify whether these imaging approaches are useful in clinical routine practice; however new MRI techniques appear promising as possible outcome measure in pharmacological clinical trials in SMC.

In conclusion our data confirm the presence of muscle fatty changes in the majority of the patients affected by SMC, although often characterized by minimal severity and without any specific pattern of involvement. However, muscle MRI may be a useful tool for longitudinal follow-up of SMC patients, in particular to evaluate the occurrence and the progression of fixed myopathy.

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Novel mutations in LMNA A/C gene and associated phenotypes

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Mutations in the lamin A/C gene (LMNA) have been associated with several phenotypes ranging from systemic to prevalent of muscle, heart, skin, nerve etc. More recently they have been associated with dilated cardiomyopathy (DCM) and severe forms of arrhythmogenic right ventricular cardiomyopathy (ARVC). We report four novel mutations - 3 missense and 1 deletion – in 4 unrelated patients showing different phenotypes, ranging from the early onset congenital form of laminopathy to classical LGMD phenotype, to LGMD and heart involvement. All these newly identified variants were not found in 300 ethnically-matched control subjects.

The variant c.103-105del CTG was described post-mortem in a young patient with congenital muscular dystrophy who presented at the age of 9 a first degree A-V block and subsequently several episodes of supraventricular parossystic tachycardia. Two patients presented as onset symptom lower limbs muscle weakness, and developed heart conduction defects requiring pacemaker implantation at the age of 26 and 38 years, respectively. One of them who carried the mutation c.1339G>C died at the age of 40 by intractable heart failure; the second one carrying the mutation 265C>T died at the age of 30, for a trmboembolic event. A classical LGMD phenotype without heart involvement was observed in the patient with the mutation 1579C>T, who died at the age of 68 years for respiratory insufficiency.

Key words: LMNA A/C gene, lamin A/C, Emery-Dreifuss muscular dystrophies, Laminopathies

Introduction

Laminopathies are a heterogeneous group of genetic disorders caused by mutation in LMNA gene encoding A and C lamins. Lamins are ubiquitous nuclear intermediate filament proteins that form a scaffold, termed nuclear lamina, at the nuclear periphery. Binding to a growing number of nuclear protein complexes, they provide nuclear stability, help connect the nucleus to the cytoskeleton, and contribute to modulate chromatin organization, gene regulation and expression, genome stability, differentiation, and tissue-specific functions (1-3).

Alterations of the nuclear envelope have been associated with several disorders including autosomal dominant (AD) forms of Emery-Dreifuss muscular dystrophy (EDMD2), dilated cardiomyopathy with conduction system defects (DCM-CD), limb girdle muscular dystrophy 1B (LGMD1B) with atrioventricular conduction disturbances, Dunnigan-type familial partial lipodystrophy (FPLD), mandibuloacral dysplasia (MAD), autosomal recessive (AR) forms of axonal Charcot-Marie-Tooth (ARCMT2,CMT2B), as far as progeroid syndromes (4-13).

To date, more than 450 LMNA mutations have been reported in locus-specific databases (http://www.umd.be/LMNA/;http://www.dmd.nl/), but few genotype/phenotype correlations have been defined, suggesting the presence of genetic modifiers. For this reason laminopathies represent a good example of "allelic heterogeneity" as mutations in the same gene can cause different phenotypes according to the site of the mutation along the gene (14, 15).

Of particular interest for cardio-myologists are the groups of LMNA-related myopathies (LM) and cardiomyopathies (LCM). LMNA-related myopathies (LM) repre-

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Table 1. Clinical features of the reported patients.

Patient	Muscle phenotype	Cardiac phenotype	Other signs	Gene Mutation	Protein	LMNA A/C exon
1	Congenital	Yes	Dropped head	c. 103-105del CTG	L35del	1
2	Mild	Yes		c.265C>T	R89C	1
3	Severe	Yes		c.1339G>C	E447Q	7
4	Severe LGMD	No	Respiratory failure	c.1579C>T	R527C	9

sent the more consistent subgroup of diseases due to mutations in LMNA gene. Three main different phenotypes have been reported based on distribution of muscle weakness or age at onset: LGMD1B, EDMD2, and a form of congenital muscular dystrophy (MDCL) (16, 17). A considerable clinical overlap exists among the three phenotypes suggesting they should be considered as a continuum in the clinical spectrum. Indeed, heart is involved in all three entities, with similar features (16, 18). Interestingly, heart involvement may precede onset of muscle weakness or sometimes be isolated. The phenotypic spectrum of muscle laminopathies ranges from severe congenital forms of muscular dystrophy to limb-girdle forms with adult onset and much milder weakness, often associated to a high risk of cardiovascular morbidity and mortality (19-22).

Here we described four novel mutations in LMNA gene in 4 unrelated patients showing phenotypes ranging from an early onset congenital muscular dystrophy to severe classical LGMD phenotype.

Patients and methods

All the reported patients are or were followed at the Cardiomiology and Medical Genetics of the Second University of Naples, between the last 10 years. All patients showed pronounced cervical muscle weakness, elbow retractions and elevation of serum creatine kinase, suggesting a Emery-Dreifuss muscular Dystrophy phenotype. The clinical features of the five patients are summarized in Table 1.

Blood samples for genetic analysis were collected after informed consent of patients or their tutors when minors, according to the Declaration of Helsinki. DNA was extracted following the standard operating procedures and analyzed trough PCR analysis of the entire coding region of LMNA A/C gene.

Results

Patients

Patient 1.

Patient 1 came to our observation for gait disturbances and frequent falls by the age of 3 years. Clinical examination revealed elbow, knee and heel retractions, and generalized muscular atrophy. until he lost ambulation by the age of 10 years for a delay in the acquisition of motor milestones. Ambulation was acquired at the age of 18 months.

Muscle biopsy showed a dystrophic pattern with a positive staining for LAMA2. From the age of 3 years to the age of 9 years he was stable. At the age of 9 years, he presented a first degree AV block and several episodes of supra-ventricular paroxysmal tachycardia, despite the treatment with beta-blockers. He died few months after for a sustained supraventricular tachycardia. The variant c.103-105del CTG (L35del) in LMNA A/C gene was found post-mortem.

Patient 2.

Patient 3 presented lower limbs muscle weakness as onset symptom, since the age of 12 years. Clinical examination revealed elbow retractions, gait disturbances and proximal muscle atrophy. Clinical Emery Dreifuss muscular dystrophy was diagnosed and LMNA A/C analysis showed the mutation c.265C>T (R89C) in exon 1. He developed heart conduction defects (hyperkinetic arrhythmia), requiring pacemaker implantation at the age of 26 years; he presented heart failure at the age of 30 and died for a tromboembolic event.

Patient 3.

Patient 2 presented since the age of 8 years a progressive weakness and gait disturbances. Elbow retractions were found at clinical examination as well as atrial fibrillation on ECG. ECG Holter revealed the presence of sinuatrial pauses, requiring pacemaker implantation at the age of 38. He lost ambulation at the age of 39 years and died at the age of 40 years by intractable heart failure. The mutation c.1339G>C (E447Q) in the LMNA gene, changing a glutamate in glutamine, at 447 position, was found.

Patient 4.

Patient 4 presented the classical phenotype of a limb girdle muscular dystrophy, at his first examination at our Service, at the age of 50. He complained of lower limbs

weakness by the age of 21, with progressive worsening in the daily motor performance. The loss of ambulation occurred at the age of 40. Clinical examination revealed proximal muscle wasting and weakness. No heart involvement was present and death occurred at the age of 68 years for respiratory failure. Later, a mutation analysis of the LMNA gene disclosed the novel missense mutation c.1579C>T, and we diagnosed him as EDMD2 (laminopathy).

Genetic analysis

All the reported mutations were novel, not previously described (transcript isoform LMNA-004, corresponding to Ensemble transcript ENST00000368299) and not found in 300 normal individuals.

The deletion occurring in the first exon of the transcript (103-105 del) determines the loss of the leucine at position 35, which is fundamental for the folding of the mature protein and the formation of the intermediate filament protein. The C to T transition (265 C>T) on the same exon determines the substitution of an arginine with a cysteine lying in the myosin rod fragment domain (R89C).

The G to C transversion (1339 G>C) and C to T transition (1579 C>T) in exons 7 and 9 respectively, determine changes in the aminoacidic composition of the Lamin A/C globular tail domain (E447Q and R527C) (Fig. 1).

Furthermore we translated the mutated transcript into its corresponding protein and annotated the functional domains through the InterProScan software (Mitchell A et al, 2015. Nucleic Acids Research).

We found that the deletion of the leucine at position 35 in particular, produces a mutated protein with the complete loss of the 30-62 SSF64593 domain which is part of the coiled coil region (Fig. 2) necessary for the structural support of the nucleus.

Discussion

Primary laminopathies caused by mutations in the LMNA gene typically display an extremely pleiotropic clinical presentation including cardiac, muscular and metabolic phenotypes. Additionally, many atypical laminopathies have been described combining features of two or more of the distinctive disorders or syndromes associated with LMNA mutations (15, 18).

Arrhythmic disorders are infrequent in young adults

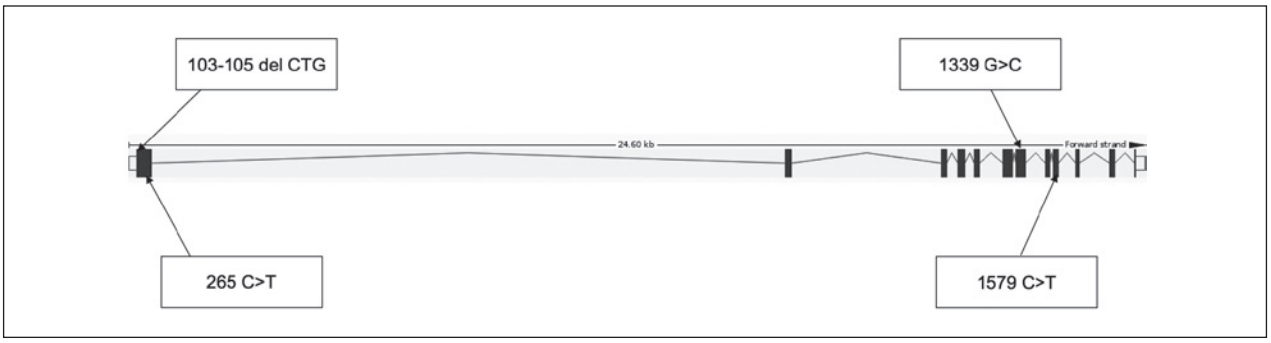

Figure 1 . Schematic representation of the LMNA A/C gene and position of novel mutations.

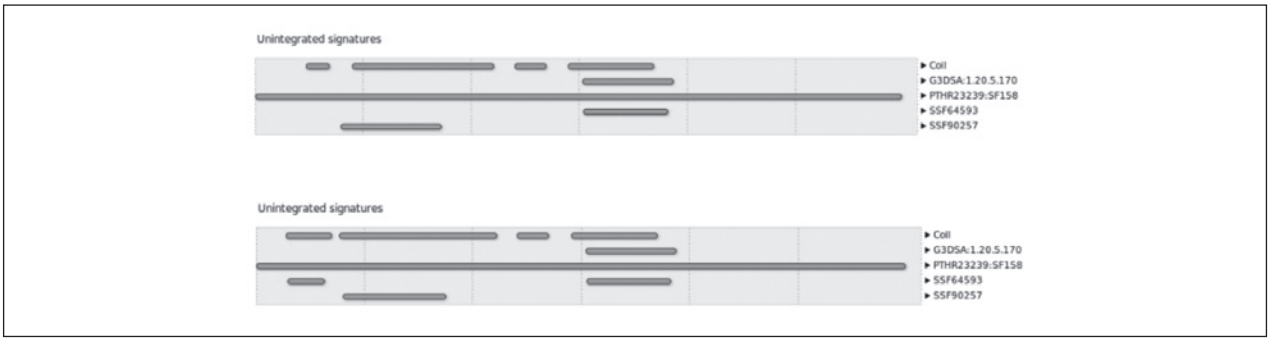

Figure 2. Comparative wild and mutated Lamin protein domains. Protein domains harboring the mutation L35del (top) and protein domains of LMNA-004 transcript (bottom). It is possible to note that some domains are missed in the mutated protein (see text). Domain annotations follows InterPro database nomenclature.

and should evoke myopathy associated cardiomyopathy, even though muscular symptoms are moderate or absent (23). Cardiac involvement is responsible for syncope, thromboembolic events and sudden death and often requires early cardioverter defibrillator implantation (24, 25).

Dropped head in children and contractures could be the clue to diagnose EDMD and indicate the need for a careful cardiological evaluation (26-29).

The reported cases further expand the role of the LMNA A/C gene in the pathogenesis of cardiac laminopathies, suggesting that LMNA should be included in mutation screening of all patients with droppen head and/or suspected arrhythmogenic cardiomyopathy, particularly when they have ECG evidence for conduction defects.

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A multi-parametric protocol to study exercise intolerance in McArdle's disease

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McArdle's disease is the most common metabolic myopathy of muscle carbohydrate metabolism, due to deficiency of myophosphorylase and alteration of glycogen breakdown in muscle. The clinical manifestations usually begin in young adulthood, with exercise intolerance, exercise-induced muscle cramps, pain and recurrent episodes of myoglobinuria. Many patients experience the *second wind* phenomenon, characterized by an improved tolerance for aerobic exercise approximately after eight minutes of motor activity, secondary to the increased availability of blood glucose and free fatty acids associated to an enhanced glucose uptake by muscle cells. In this study, we aimed to test a multi-parametric protocol in order to detect the impairment of muscular metabolism and motor performance in patients with McArdle's disease. We enrolled 5 patients and 5 age-matched healthy subjects, that were evaluated by: (1) monitoring of physical activity with an electronic armband; (2) testing of cardiopulmonary, metabolic and respiratory responses to exercise with a cardiopulmonary exercise test and analyzing muscle fatigue during exercise test by surface electromyography (4) evaluating blood lactate and oxidative stress biomarkers at rest and during exercise. The patients were tested at baseline and after three days of carbohydrate-rich diet integrated with tricarboxylic acid cycle intermediate and creatine. The multi-parametric protocol proved to be useful to detect the oxidative capacity impairment and the *second wind* phenomenon of patients. We did not observe any significant differences of muscle metabolic response during the exercise test after three days of carbohydrate-rich diet.

Key words: McArdle disease, muscle exercise, myophosphorylase deficiency

Introduction

McArdle disease, or glycogen storage disease type V (GSDV, OMIM #232600), is the most common metabolic myopathy of muscle carbohydrate metabolism, with a prevalence of 1 in 100000 individuals (1), caused by homozygous or compound heterozygous mutations in the *PYGM* gene on chromosome 11q13, which encodes muscle glycogen phosphorylase (2, 3). The myophosphorylase deficiency is responsible of the alteration of glycogen breakdown in muscle, which is an important fuel for the contraction of muscles, especially in prolonged exercise at high intensities.

The clinical symptoms of GSDV usually begin in young adulthood with exercise intolerance, exercise-induced muscle cramps, pain and recurrent episodes of myoglobinuria, which may lead to acute renal failure. Many patients experience the *second wind* phenomenon, characterized by an improved tolerance for aerobic exercise approximately after seven to eight minutes of motor activity. The *second wind* phenomenon is determined by the increased availability of blood glucose and free fatty acids associated to an enhanced glucose uptake by muscle cells (2, 3).

Several dietary and pharmacological treatments have been tested to alleviate symptoms in this disease, but most of them failed to demonstrate a significant

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amelioration or were not well tolerated. The previous studies have often included a small number of patients; furthermore the clinical heterogeneity of GSDV might make it difficult to establish measurable primary outcomes (4, 5).

The aim of our study was to define a multi-parametric evaluation protocol in patients with GSDV in order to detect and quantify the impairment of both muscular energetic metabolism and motor performance. The protocol has been subsequently tested to investigate the effect of a carbohydrate-rich diet integrated with tricarboxylic acid cycle intermediate and creatine on exercise intolerance and motor skills in the same patients.

Material and methods

Patients recruitment

We enrolled 4 patients affected by GSDV (1 female, 3 males; age ranging from 19 to 58 years), including two brothers and two unrelated patients. All patients had experienced life-long exercise intolerance, repeated exercise-induced episodes of muscle cramps and occasional myoglobinuria. The diagnosis was confirmed by genetic and/or biochemical testing on muscle biopsy (Table 1). We also enrolled a female patient (patient 5, Table 1) reporting exercise intolerance that presented a partial myophosphorylase deficiency and was heterozygote for the mutation R50X in *PYGM* gene. A control group of 5 sex- and age-matched healthy subjects was included. Any subjects took medication at the time of the study. All patients and healthy subjects were informed of the risk and nature of the study and gave consent to participate.

Multi-parametric evaluation protocol

Pre-experimental preparations

Both patients and healthy subjects, following a free diet, were continuously monitored for three days with a metabolic holter SenseWear Armband. Patients and controls underwent to exercise protocol on a bicycle ergometer (described above). Afterwards the patients followed a carbohydrate-rich diet integrated with tricarboxylic acid cycle intermediate and creatine (6) for three days, during which they were monitored again with the armband. The composition of the carbohydrate-rich diet was 20% fat, 15% protein and 65% carbohydrate (vegetarian food: vegetables, fruits, pasta, rice, bread and low-fat cheese). The amount of the calories in the diet was adjusted to the subject's weight, age and sex. The caloric intake was on average 2700 kcal for men and 2200 kcal for women. Each patient was instructed about the diet by a nutritionist. During the carbohydrate-rich diet patients also took 4 capsules/day of a food supplement called "CREATINE STRONG MATRIX 7", containing creatine malate, creatine ethyl ester HCL, creatine alpha ketoglutarate, creatine orotate, creatine pyruvate, creatine citrate. As one capsule contains 1.22 g of creatine, the used daily dose of creatine resulted safe, according to previous reports (7, 8). Then, after the three days of diet, the patients repeated the test on the bicycle ergometer.

Metabolic Holter

Daily physical activities was objectively measured using a validated multisensory array, the BodyMedia SenseWear Armband (9). SenseWear Armband is a wearable device that utilizes a 2-axis accelerometer, heat flux sensor, galvanic skin response sensor (GSR), skin temperature sensor, and a near-body ambient temperature sensor to capture data leading to the calculation of energy expenditure (9). In this study we used Armband to measure: Basal Metabolic Rate (BMR), Total Energy Expendi-

Table 1. Patients enrolled in the study.

Patients	Age (years)	Sex	BMI	Myophosphorylase staining muscle biopsy	Genetic test PYGM gene
Patient 1*	23	Male	26,78	Absent	Homozygous mutations R50X
Patient 2*	19	Male	25,25	Absent	Homozygous mutations R50X
Patient 3	58	Male	30,07	Absent	--
Patient 4	34	Female	24,09	Absent	--
Patient 5	39	Female	19,15	Reduced	Heterozygous mutations R50X

(*brothers)

ture (TEE), number of average METs (1 MET=1 Kcal/kg/hour; a normal healthy subject has a BMR of 1 MET, and positive or negative deviations from this value suggest respectively a hypermetabolic or a hypometabolic condition), number of steps, Physical Activity Duration (PAD), Active Energy Expenditure (AEE).

The device was worn by the enrolled subjects continuously for 72 hours during daily activities and during the exercise protocol.

Exercise test protocol

All subjects were tested between 9 and 10 a.m; they had breakfast 2-2½ hours before exercise testing. They performed a cardiopulmonary exercise test (CPET) on a cycle ergometer at a constant workload of 50% of VO_2 max for 12 minutes, followed by an incremental test until exhaustion, using increments in workload of 15 watts every two minutes. The constant workload test was used to evaluate the occurrence of the *second wind* phenomenon, the heart rate and the rating of perceived exertion. The incremental test was used to determine the maximal oxidative capacity. The level of perceived exertion was scored every minute, using a visual analogue scale (Borge scale, 10). Cardiopulmonary, respiratory and metabolic responses to exercise were monitored continuously through the measurement of VO_2, VCO_2, RER, VE/VO_2, VE/VCO_2, $PETO_2$, $PETCO_2$, HR, O_2 pulse (see the legend).

Blood samples were obtained at rest, exercise peak and recovery to analyse plasma lactate and oxidative stress biomarkers such as advanced oxidation proteins products (AOPP) and thiols (11, 12).

Furthermore, during exercise, a surface electromyography (sEMG) monitoring on quadriceps femoris muscle using wireless platform was performed , in order to analyze the sEMG signal during dynamic contractions and to study the muscular activity (13). sEMG technology is a non-invasive and non-painful analysis that allows information regarding the overall muscle function and condition collected from the surface of the skin. After the signal acquisition and filtering for reduction of artifacts, the energy values, expression of the motor units recruitment, for each 20 seconds after full wave rectification and smoothing using a low pas filter 2.4Hz (13) were extracted.

Statistical analysis

Differences between patients and controls, at baseline and after dietetic treatment, were assessed by a Student's t-test; an analysis of variance was used to test whether significant changes in measured variables occurred with time. A p value < 0,05 was considered statistically significant.

Results

Metabolic Holter at basal conditions during a free dietary regimen

BMR resulted higher in controls then in patients. Also TEE was higher in controls then in patients, especially in terms of AEE. According to these data, the daily number of steps is on average 13253 in controls, whereas in patients is less then 10000, indicating a very low daily physical activity.

Even the average METs was higher in controls (1,6) then in patients (1,4) (Table 2).

Metabolic Holter during carbohydrate-rich diet integrated with tricarboxylic acid cycle intermediate and creatine

No significant difference between baseline and the three days diet treatment was observed in the patients group during daily activities.

Exercise protocol performed during a free dietary regimen

During the constant workload exercise on the bicycle ergometer, 3 patients experienced the characteristic second wind phenomenon with a peak heart rate of 143,3 bpm at 8 minute of exercise (Figure 1) and one patient was unable to complete the exercise because of the onset of cramps and fatigue. In the patient with partial myophosphorylase deficiency (heterozygous carrier, patient 5) the heart rate increased progressively during the exercise, as in healthy subjects. Heart rate was consistently lower in healthy subjects then in patients ($p < 0.05$), suggesting they were performing at a higher percentage of their exercise reserve. During the incremental workload, the patients managed to reach lower

Table 2. Comparison of average values of holter metabolic monitoring between patients and heathy controls.

	BMR (cal)	BMR (METs)	TEE (cal)	PAD (hours)	AEE (cal)	Steps number	Sleeping duration (hours)	METs average
Patients	1565	0,88	2345	1:05	292	7488	6:35	1,4
Controls	1565	0,94	2683	2:23	605	13253	6:17	1,6

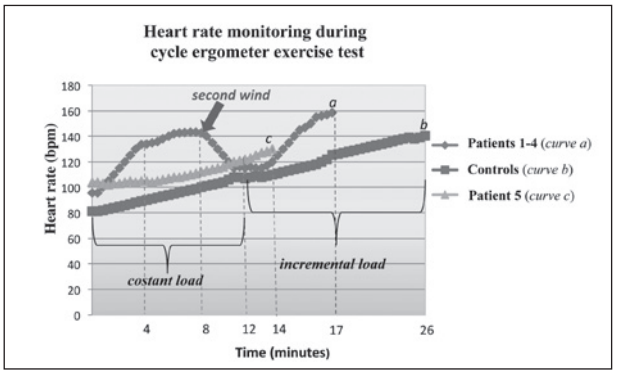

Figure 1. Heart rate monitoring during cycle ergometer exercise test.

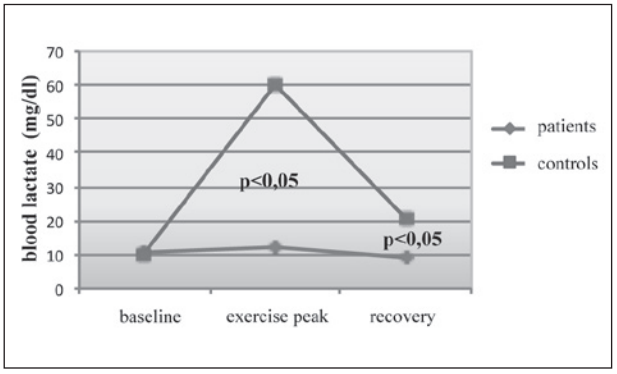

Figure 2. Blood lactate levels during exercise test.

maximal workload levels compared to healthy subjects, with a higher score of perceived exertion and higher levels of heart rate. The perceived exertion reflected the heart rate trend.

Both VO_2 max and VCO_2 max were significantly lower in patients ($p < 0,05$), according to the oxidative capacity impairment observed in McArdle's disease. RER value was constant and < 1 in patients, while increased progressively to 1 value in controls, indicating the inability to use glycogen by muscle during sustained exercise, in McArdle's disease.

The monitoring of the muscle activity by sEMG during costant workload and incremental exercise on the bicycle ergometer showed that the value of energy extracted from sEMG signal analysis was higher in patients then in healthy subjects ($p < 0,03$), suggesting they were performing the exercise at a higher percentage of their motor reserve.

Exercise protocol performed after the carbohydrate-rich diet integrated with tricarboxylic acid cycle intermediate and creatine

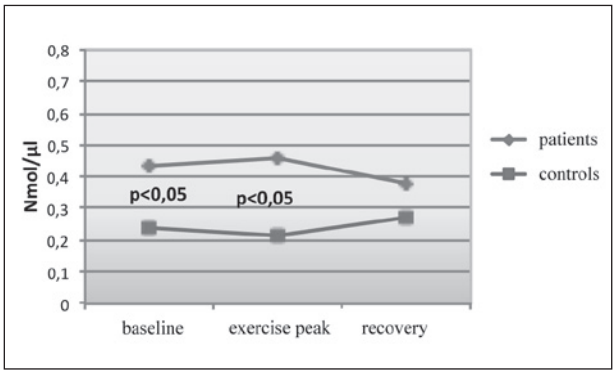

Figure 3. Thiols level during exercise test.

No significant difference between baseline and the three days dietary treatment was observed in the group of patients.

Blood lactate

- *At basal conditions during a free dietary regimen.*

The typical "flat curve" was observed in the patients, as shown in Figure 2.

- *After carbohydrate-rich diet integrated with tricarboxylic acid cycle intermediate and creatine.*

No significant difference between baseline and the results obtained after three days of carbohydrate-rich diet integrated with TCA intermediates and creatine was observed.

Oxidative stress biomarkers

- *At basal conditions during a free dietary regimen.*

The analysis of blood oxidative stress biomarkers at exercise rest, peak and recovery showed significantly higher levels of thiols in patients then in healthy subjects, at basal condition and at peak of exercise ($p<0.05$) (Figure 3). Lower levels of AOPP in patients than in healthy subjects were also observed, although this difference did not reach a statistical significance.

- *After carbohydrate-rich diet integrated with tricarboxylic acid cycle intermediate and creatine.*

No significant differences in oxidative stress biomarkers levels during exercise performed in basal conditions and after the three days dietary treatment were observed in the patients group.

Discussion

In this study we defined a multi-parametric evaluation protocol in patients with GSDV in order to characterize the impairment of the muscle metabolism and motor performances. Because a previous report observed a ben-

eficial effect of a carbohydrate-rich diet compared to a protein-rich diet (14), we also investigated the effect of a carbohydrate-rich diet integrated with tricarboxylic acid cycle intermediate and creatine, on motor performance and exercise intolerance in these patients.

The monitoring with metabolic holter was able to record a lower level of daily physical activity in patients with McArdle disease, according to their clinical manifestations, such as muscle fatigue and exercise intolerance. A daily activity monitoring in GSDV patients could be useful to test the effect of aerobic training to improve motor performance (15).

GSDV patients should avoid a sedentary lifestyle, which induces deconditioning, and engage in regular and moderate aerobic exercise, in order to increase the circulatory capacity and the delivery of blood-borne fuels, inducing a sort of "permanent *second wind*" (1, 15).

The exercise protocol on a cycle ergometer used in this study has been previously proposed in GSDV patients (6). In our study, the CPET on a cycle ergometer confirmed the impairment of both anaerobic and oxidative metabolism in GSDV patients skeletal muscle. The anaerobic metabolism impairment was documented by the typical lactate "flat" response (16). The analysis of cardiopulmonary parameters evidenced that in GSDV patients VO_2 max and VCO_2 max resulted significantly lower according to the altered oxidative capacity. The inability to use glycogen by muscle during sustained exercise in McArdle disease is confirmed by the respiratory exchange rate (RER) value, an indirect index of the different substrate utilization: RER value resulted <1 and constant during exercise in GSDV patients, while increased progressively to 1 in the controls.

The constant workload exercise test was so sensitive to appreciate the *second wind* phenomenon in GSDV patients. The exercise protocol was also able to identify the pattern of the heterozygous carrier, who showed intermediate phenotypic characteristics between patients and healthy subjects. In this patient the partially reduced myophosphorylase activity may explain the symptoms - such as exercise intolerance, cramps and myalgias – occurring during sustained efforts, but proves to be enough to allow glycogen utilization during a prolonged exercise (17). According to that, the heart rate during the test increased progressively as in all the healthy subjects in the heterozygous carrier and the second wind phenomenon was not revealed (17).

Interestingly, in the patients with GSDV here reported, increased levels of oxidative stress in basal condition and during exercise test were not apparently observed, as deduced by reduced levels of thiols ($p < 0.05$) at basal condition and at peak of exercise in patients compared to controls.

Considering the defect of oxidative metabolism in McArdle disease, it could be supposed that the higher levels of thiols detected in our patients could be due to the low flow of substrates through the TCA cycle which limits the oxidative capacity, oxygen consumption and oxidative phosphorylation in mitochondria and consequently the physiological production of reactive oxygen species (ROS) in basal condition and during the motor activities.

The impairment of muscle mitochondrial respiration has been previously shown with ^{31}P-MR spectroscopy (18). However these data should be confirmed in a larger cohort of patients. Anyway, it should be also consider that the duration of exercise effort was on average minor in patients then in healthy subjects and that the amount of ROS produced during exercise is proportional to its duration.

Finally by the same protocol the effect of three days of carbohydrate-rich diet integrated with tricarboxylic acid cycle intermediate and creatine was investigated. In this respect, partially conflicting evidences on significant benefits from vary specific nutritrional treatment in GSDV are reported in literature. It has been hypothesized that a carbohydrate-rich diet might improve exercise intolerance in GSDV patients by maintaining high glycogen stores in the liver. This hypothesis is supported by the key role of blood glucose for generating the second wind phenomenon, caused by an enhanced uptake and oxidation of glucose and, to a smaller extent, fatty acids (2). The mobilization of hepatic glucose is exaggerated during exercise in patients with GSDV because of a higher sympatho-adrenal response that is brought about by the initial energy crisis early in exercise. The glucose resulting from hepatic glycogenolysis results crucial for partially compensation of the blocked muscle glycogenolysis (14, 19). Previous single-case studies have also suggested that a protein-rich diet could be beneficial in GSDV patients (20, 21). In a previous cross-over open-design study (14) a carbohydrate-rich diet (20% fat, 15% protein, 65% carbohydrate) was proved to increase the maximal work capacity and exercise tolerance of submaximal workload in comparison with protein-rich diet (15% fat, 55% protein, 30% carbohydrate) in 7 GSDV patients. However, the authors suggested that other trials were needed to confirm the effect of carbohydrate-rich diets in GSDV, also by comparing with non-protein-rich diets and assessing the long-term effects (14).

In our study, the effect of a carbohydrate-rich diet on motor performance and exercise intolerance in 4 GSDV patients was analyzed by comparing the results obtained in basal conditions and after the diet, integrated with TCA intermediates and creatine, in order to improve oxidative capacity in these patients. In fact, the limited glycolysis in GSDV during exercise inevitably produces low concentrations of TCA cycle intermediates (22).

Using the same multi-parametric exercise protocol, we did not observed any differences between basal condition and three days diet; only one patient referred a mild subjective benefit in daily activities during the diet, with reduced fatigability. These results are apparently disagree with the ones described by Andersen and Vissing in 2008 (14), although the latter study reported a benefit of carbohydrate-rich diet only in comparison with a protein-rich one. Notably, several lines of evidence suggest that extra protein should not be helpful in McArdle disease. In fact, amino acids, the constituent of proteins, play a minor role in muscle energy metabolism during exercise, which is covered almost exclusively by fat from adipose tissue and by carbohydrates derived from hepatic and muscle glycogen stores (23). Therefore, we hypothesize that the absence of benefit of carbohydrate-rich diet observed in our study may be due to the comparison with a baseline condition and no with a protein enriched diet, that can be deleterious on motor performances in GSDV. It is also important to note that the Mediterranean diet, daily followed by our patients, is *per se* a carbohydrate-rich diet, with percentage of macronutrients very similar to the ones of the diet followed by patients during the three days of treatment and it is by itself beneficial in these patients.

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Legend

VO_2 = oxygen uptake
VCO_2 = CO_2 production
RER = respiratory exchange ratio
VE/VO_2 = ventilatory equivalents for O_2
VE/VCO_2 = ventilatory equivalents for CO_2
$PETO_2$ = end-tidal O_2
$PETCO_2$ = end-tidal CO_2
HR = heart rate
O_2pulse = Oxygen pulse (oxygen uptake per heartbeat at rest)

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Multidimensional aspects of pain in myotonic dystrophies

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To analyze the frequency and intensity of pain and its association with different characteristics of patients with myotonic dystrophy type 1 (DM1) and type 2 (DM2), 52 adult genetically confirmed DM1 and 44 DM2 patients completed the Brief Pain Inventory questionnaire (BPI).

Frequency and average intensity of pain on numerical rating scale (0-10) were similar in DM1 and DM2 (88% vs. 86% and 4.6 ± 2.3 vs. 4.2 ± 1.8, respectively, $p > 0.05$). In DM1, average pain intensity showed strong association with longer duration of disease and inverse relation with cognition. In DM2, average pain intensity showed association with female gender and emotions. Average pain intensity correlated with Individualized Neuromuscular Quality of Life (INQoL) total score in both DM1 (rho = +0.30, $p < 0.05$) and DM2 patients (rho = +0.61, $p < 0.01$).

In conclusion, the majority of DM1 and DM2 patients have mild to moderate pain. Our results open new opportunities for behavioral and cognitive interventions.

Key words: myotonic dystrophy type 1, myotonic dystrophy type 2, pain, quality of life

Introduction

For many years inherited neuromuscular disorders were considered painless. In the last ten years plenty of evidence suggested that pain was very common and even one of the core features of certain diseases from this group (1-6).

Myotonic dystrophies are the most common inherited muscular disorders in adults (7). They are autosomal dominant diseases caused by pathological expansion of nucleotide repeats – CTG repeats in the DMPK gene in case of myotonic dystrophy type 1 (DM1), and CCTG repeats in the CNBP gene in case of myotonic dystrophy type 2 (DM2) (7).

Pain is present in about 50-80% of DM2 patients, being one of the main features of the disease with potential impact on quality of life (QoL) (8-10). In 11% of DM2 cases it may even be the first symptom of the disease (11). Pain is often overlooked in DM1 both by patients and physicians, who often have a greater focus on other symptoms, such as weakness, myotonia, heart problems and anesthesia risk. Such limited focus on pain may impact patient care, as other studies have reported high levels of pain in 45-77% of DM1 patients with its possible influence on QoL (1, 2, 4-6, 10, 12-14).

To the best of our knowledge, direct comparison of the pain features in patients with DM1 and DM2 has not been performed so far. Furthermore, myotonic dystrophies are multisystemic disorders affecting different organs including the central nervous system with different cognitive and behavioral impairments (7). These diseases also have an impact on social participation (15). Previous studies usually assessed pain in DM1 patients together with patients with other neuromuscular disorders and investigated pain influence only on certain areas of life (1, 5). Number of pain studies in DM2 is even smaller giving only descriptive data of pain and its influence on QoL (9, 10, 16). Separate analysis of each type of DM and a comprehensive approach investigating different biological, behavioral, cognitive, and social aspects would define the most significant factors and help to eliminate confounding variables related to pain in these multisystemic diseases.

The aim of the study was to analyze frequency and

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intensity of pain and its association with different characteristics of patients with DM1 and DM2.

Patients and methods

Approval for this cross sectional study was received from the Ethics Committee of the School of Medicine, University of Belgrade, and written informed consent was obtained from all subjects participating in the study. Adult DM1 (n = 52) and DM2 (n = 44) patients were consecutively recruited from the Inpatient Unit and Day Hospital of the Neurology Clinic, Clinical Centre of Serbia at the University of Belgrade from January 1, 2013 until September 30, 2014. Most of them were newly diagnosed, while others were hospitalized due to the regular check-ups but not because of the worsening of symptoms. Clinical and electrophysiological diagnosis was confirmed by Repeat Primed PCR and Southern blot analysis in DM1 and by Repeat Primed PCR in DM2 patients (17). Patients with congenital, childhood and juvenile form of DM1 were excluded from the study, as well as all DM1 and DM2 patients with other significant somatic, neurological and psychiatric disorders not related to the disease; in particular 3 patients with DM1 were excluded due to acute stroke, leg fracture and severe depression with suicide attempt respectively, and 4 patients with DM2 associated with other diseases (neuromyelitis optica, leg amputation in non-regulated diabetes, leukemia, and acute heart infarction).

Pain was investigated by the shorter version of the Brief Pain Inventory questionnaire (BPI) (18) since questions about pain interference were not analyzed in this study. Patients were asked if they, during the last four weeks (instead of the last 24 hours in the original questionnaire), had any pain or pain other than everyday kinds of pain seen even in healthy people from time to time. They were also asked to draw their sites with pain on body map, and to rate experienced pain on 0-10 numerical rating scale at its worst, least, and average in the last four weeks. They answered what pain medication they used and rated pain relief on 0-10 numerical scale.

Since the Muscular Impairment Rating Scale (MIRS) for severity of muscular involvement is applicable only in DM1 and not in DM2, manual muscle testing (0 to 5 scale according to Medical Research Council (MRC) scale) was performed by experienced neurologists (VRS, SP, DL, IB). We added strength of the weakest muscle of the proximal arms, distal arms, proximal legs and distal legs in both DM1 and DM2 patients, with maximum score being 20 (19). Duration of active hand grip myotonia and percussion myotonia of the thenar eminence was measured in seconds.

Patients completed the Individualized Neuromuscular Quality of Life questionnaire (INQoL) (20, 21). INQoL consists of 45 questions within 10 sections. Four sections measure the impact of common muscle disease symptoms (weakness, locking (aka myotonia), pain and fatigue). Five sections measure the influence of the muscle disease on particular areas of life (activities, independence, relationships, emotions and body image). The last section is related to disease treatment and it was not used in our study. Total INQoL score is calculated from five sections assessing the influence of the muscle disease on particular areas of life according to Vincent et al. (20). The final score for each of nine sections and total INQoL score is presented as a percentage of the maximum detrimental impact with a higher percentage indicating greater symptom impact or worse QoL.

Depressive symptoms were assessed with the Hamilton rating scale for depression (HamD) (22). Severity of fatigue was measured by the Krupp's Fatigue Severity Scale (FSS) (23). The daytime sleepiness scale (DSS) was administered to all patients (24). In further text depression, fatigue and excessive daytime sleepiness were referred to as behavioral factors.

Educational level was measured as number of years spent at school. All patients underwent neuropsychological investigation performed by experienced neuropsychologists. Global cognitive status was assessed using the Mini Mental State Examination (MMSE) with score of ≤ 24 considered abnormal (25). No one of tested patients had MMSE ≤ 24, i.e. suspected dementia. For assessment of delayed verbal memory, the Rey Auditory Verbal Learning Test (RAVLT) was administered (26). Copy and recall of the Rey-Osterrieth Complex Figure (ROCF) was used to assess visuo-constructive abilities and visual memory (26). Speed and attention were assessed using the Trail Making Test A (TMT-A) (26). Executive functions were examined by the Trail Making Test B (TMT-B) (26). Tests were chosen in order to measure general cognitive level and major neuropsychological domains (visual ability, verbal and visual memory, attention and executive functions). All these tests have been widely used in patients with DM1 and DM2, including our cohorts (13, 19). Higher scores on MMSE, RAVLT and ROCF, and lower scores on TMT-A and TMT-B imply better cognitive achievement.

Normality of data was tested by the Kolmogorov-Smirnov test. For comparison between two groups χ^2 test, Mann-Whitney U test and Student t test were used, as appropriate. Correlations were calculated using nonparametric Spearman's coefficient. Factors that significantly correlated with average pain intensity in the last four weeks were included in the first linear regression analyses. Four separate multivariate linear regression analyses were used for each group of independents (1. demograph-

ic and clinical, 2. behavioral, 3. cognitive, and 4. social). In this way, number of covariates in the final regression analysis was reduced. Final multivariate linear regression analysis encompassed all significant predictors from the first regression analysis to identify the factors with the strongest association with average pain intensity. At the both levels of regression analysis stepwise method was used – we entered all the univariately significant effects and SPSS made stepwise inclusion to fit the best model with the probability of F to enter ≤ 0.05 and to remove ≥ 0.10. Using two levels of linear regression analysis all confounding variables and false positive findings were excluded. In all statistical analyses, significant testing was two-sided, with alpha set at 0.05 for statistical significance and 0.01 for high statistical significance.

Results

Frequency of any pain was similar in DM1 and DM2 (88.5% vs. 86.4%, $p > 0.05$) (Table 1). Frequency of pain other than everyday kinds of pain was even somewhat higher in DM1 patients but without statistical significance (51.9% vs. 38.6%, $p > 0.05$). The most common pain sites were lumbosacral spine (42.3%) and lower legs (34.6%) in DM1, and lower and upper legs (40.9% and 29.5%, respectively) in DM2. Intensity of pain was similar in both groups ($p > 0.05$). Analgesics were used in 46.2% of DM1 patients vs. 47.7% of DM2 patients ($p > 0.05$): non steroid anti-inflammatory drugs were used in 42.3% vs. 29.5%, paracetamol in 3.8% vs. 13.6%, and neuropathic pain medication (gabapentin or pregabalin) in 1.9% vs. 13.6%.

Table 1. Frequency, sites and intensity of pain in patients with DM1 and DM2.

Feature	DM1 (n = 52)	DM2 (n = 44)
Frequency of pain		
Usual pain (%)	88.5	86.4
Unusual pain (%)	51.9	38.6
Pain sites		
Head *	7.7	25.0
Neck	3.8	0.0
Shoulders	17.3	15.9
Upper arms *	7.4	22.7
Lower arms	9.6	15.9
Hands	11.5	13.6
Trunk	3.8	4.5
Lumbosacral spine *	42.3	22.7
Upper legs *	13.5	29.5
Knees	17.3	15.9
Lower legs	34.6	40.9
Feet	7.7	11.4
Number of pain sites	1.9 ± 2.0	2.2 ± 1.6
Pain intensity †		
Minimum	4.3 ± 2.6	3.6 ± 2.0
Average	4.6 ± 2.3	4.2 ± 1.8
Maximum	5.6 ± 2.6	4.9 ± 2.5
Analgesics		
Use (%)	46.2	47.7
Pain reduction (%)	63.4 ± 38.0	64.6 ± 34.0

* $p < 0.05$ for comparison between DM1 and DM2 patients; † measured with numeric rating scale (0-10)

The investigated demographic and clinical factors are presented in Table 2. Average pain intensity showed strong association with longer duration of disease in DM1 (beta = +0.47, $p < 0.01$), and with female gender (beta = +0.38, $p < 0.01$) and more severe muscular weakness in DM2 (beta = +0.58, $p < 0.01$).

In patients with DM1, a strong association between average pain intensity and severity of fatigue was observed (beta = +0.41, $p < 0.01$) (Table 2). In DM2 patients pain was related with the emotions subscale of INQoL (beta = +0.62, $p < 0.01$).

The achievements on neuropsychological tests are presented in Table 3. In patients with DM1 the average pain intensity was inversely associated with results on MMSE (beta = -0.46, $p < 0.05$), while in DM2 patients the strongest positive association was observed between pain and results on TMT-A (beta = +0.53, $p < 0.01$).

The social characteristics of patients are given in Table 3. Pain intensity in DM1 subjects was in association with subdomain independence from INQoL (beta = +0.41, $p < 0.01$), while in DM2 patients pain was related with INQoL subdomains independence (beta = +0.37, $p < 0.05$) and relationships (beta = +0.35, $p < 0.05$).

The results of the second multivariant linear regression analysis are presented in Table 4. The most significant factors related to pain in DM1 were duration of disease (beta = +0.35, $p < 0.05$) and MMSE (beta = -0.46, $p < 0.05$), and in DM2 female gender (beta = +0.37, $p < 0.01$) and emotions domain of INQoL (beta = +0.64, $p < 0.01$).

Mean INQoL total score was similar in both patients groups (34.2 ± 28.9 in DM1 vs. 41.6 ± 23.7 in DM2, $p > 0.05$). Average pain intensity correlated with INQoL total score in both DM1 (rho = +0.30, $p < 0.05$) and DM2 patients (rho = +0.61, $p < 0.01$).

Discussion

Pain was present in almost 90% of DM1 and DM2 patients in the last four weeks prior to testing. Pain other than everyday kinds of pain was reported by 52% of DM1 and 39% of DM2 patients. In previous studies pain was found in approximately 50-80% of both DM1 and DM2

Table 2. Association of average pain intensity with demographic, clinical and behavioral factors in patients with DM1 and DM2.

Feature	DM1	Univariate analysis (p)	Multivariate analysis (beta, p)	DM2	Univariate analysis (p)	Multivariate analysis (beta, p)
Demographic and clinical						
Females %	55.8	0.162	n.i.	68.2	0.016	+0.38, 0.002
Age at onset ** years, mean ± SD	25.3 ± 8.6	0.337	n.i.	37.4 ± 12.0	0.882	n.i.
Duration mean ± SD	19.9 ± 9.7	0.001	+0.47, 0.001	16.0 ± 13.4	0.017	+0.08, 0.548
Age ** years, mean ± SD	45.0 ± 10.7	0.194	n.i.	53.4 ± 11.1	0.006	+0.03, 0.847
MRC score mean ± SD	16.1 ± 2.6	0.017	-0.17, 0.389	17.0 ± 2.3	0.002	-0.12, 0.409
INQoL weakness mean ± SD	48.9 ± 36.8	0.049	+0.20, 0.129	60.6 ± 32.5	0.000	+0.58, 0.000
INQoL locking mean ± SD	44.1 ± 37.9	0.107	n.i.	42.3 ± 34.4	0.013	+0.18, 0.199
Active myotonia ** s, mean ± SD	5.9 ± 2.4	0.697	n.i.	1.8 ± 1.9	0.686	n.i.
Percussion myotonia ** s, mean ± SD	8.8 ± 2.4	0.282	n.i.	3.7 ± 7.9	0.408	n.i.
R^2 adjusted			0.20			0.44
Behavioral						
HamD score ** mean ± SD	13.3 ± 8.0	0.022	-0.01, 0.943	7.8 ± 8.0	0.001	+0.17, 0.211
INQoL emotions mean ± SD	29.2 ± 30.2	0.057	n.i.	32.3 ± 25.2	0.000	+0.62, 0.000
DSS mean ± SD	5.8 ± 2.9	0.076	n.i.	5.1 ± 2.8	0.462	n.i.
FSS mean ± SD	35.2 ± 13.0	0.003	+0.41, 0.003	38.2 ± 15.5	0.000	+0.32, 0.064
INQoL fatigue mean ± SD	41.9 ± 39.1	0.020	+0.18, 0.221	54.3 ± 33.8	0.000	+0.19, 0.305
R^2 adjusted			0.15			0.37

** p<0.01 when compared patients with DM1 and DM2; n.s. non-significant; n.i. not included in the multivariate analysis since it was non-significant in univariate analysis

subjects (8-10). However, it is traditionally reported that pain is more common in DM2 than in DM1 (7, 8, 11). This may be due to the fact that DM2 is less severe in terms of muscular and multisystemic affection (7), thus pain might be the foreground of the disease. One third of DM2 patients considered pain the most disabling feature of their disease (16), and it represents the first symptom of the disease in 11% of patients (11). In line with this, in 3% of patients primarily diagnosed with fibromyalgia, a final diagnosis of DM2 was established (27). Similar or even higher percentage of pain in DM1 compared to DM2 patients found in our study should alarm clinicians to think about pain management in DM1, not only in DM2.

The most common sites of pain were lumbosacral spine and lower legs in DM1 patients, and whole legs in DM2. In both types of the disease pain was mostly located in the areas associated with locomotion which might have significant association with patients' walking ability. Findings in previous studies were similar regarding the common involvement of different parts of legs (6, 7, 9, 16). Furthermore, leg pain had the highest contribution to the pain interference in DM1 (28). Lumbosacral pain was frequent in both DM1 and DM2 subjects, but more common in DM1. In previous studies, up to two third of DM1 patients had back pain (4, 6). Back pain in neuromuscular disorders including myotonic dystrophy, might be associ-

Table 3. Association of average pain intensity with cognitive and social factors in patients with DM1 and DM2.

Feature	DM1	Univariate analysis (p)	Multivariate analysis (beta, p)	DM2	Univariate analysis (p)	Multivariate analysis (beta, p)
Cognitive						
Education years, mean ± SD	11.0 ± 2.7	0.268	n.i.	11.4 ± 3.2	0.025	-0.12, 0.507
MMSE ** mean ± SD	26.0 ± 2.6	0.041	-0.46, 0.016	28.0 ± 2.4	0.172	n.i.
ROCF copy mean ± SD	21.8 ± 8.2	0.059	n.i.	19.7 ± 7.7	0.672	n.i.
ROCF recognition mean ± SD	12.6 ± 5.3	0.306	n.i.	12.0 ± 4.3	0.977	n.i.
RAVLT recognition ** mean ± SD	13.3 ± 5.4	0.219	n.i.	27.4 ± 5.7	0.007	-0.25, 0.176
TMT-A mean ± SD	61.0 ± 28.6	0.253	n.i.	61.9 ± 38.2	0.012	+0.53, 0.002
TMT-B mean ± SD	163.9 ± 61.7	0.084	n.i.	144.9 ± 78.8	0.038	+0.00, 0.992
R^2 adjusted			0.18			0.26
Social						
Marital status (%) live with partner live without partner	50.0 50.0	0.499	n.i.	32.6 67.4	0.557	n.i.
Work (%) ** manual labour intellectual unemployed retired	26.9 26.9 28.8 17.3	0.092	n.i.	34.1 18.2 6.8 38.6	0.583	n.i.
INQoL activities mean ± SD	34.8 ± 31.9	0.091	n.i.	49.9 ± 29.6	0.001	-0.01, 0.965
INQoL relationships mean ± SD	18.1 ± 24.9	0.026	+0.13, 0.493	22.0 ± 23.1	0.000	+0.35, 0.021
INQoL independence mean ± SD	32.7 ± 36.0	0.004	+0.41, 0.003	38.4 ± 32.4	0.000	+0.37, 0.017
INQoL body image mean ± SD	35.8 ± 28.3	0.024	-0.11, 0.632	34.4 ± 21.9	0.000	+0.153, 0.459
R^2 adjusted			0.15			0.37

** $p < 0.01$ when compared patients with DM1 and DM2; n.s. non-significant; n.i. not included in the multivariate analysis since it was non-significant in univariate analysis

ated with weakness of specific muscle groups. The activation of the stronger muscles is increased in order to oppose the forces acting on joints and this might cause pain and even skeletal abnormalities, including painful spinal deformities. In addition to the musculoskeletal pain, it is of mention that headache was more common in our DM2 subjects with frequency of 25% as previously reported, and as similar as in general population (9). On the other hand, significant correlation between headache and QoL was reported in DM1 patients (28). Thus, there future studies regarding different aspects of headache, and not only musculoskeletal pain, are necessary in myotonic dystrophies and other neuromuscular disorders.

Patients with myotonic dystrophies in this series had mild to moderate pain without significant differences between minimum and maximum pain intensity. Similar pain intensity was found in previous studies (1, 7, 9, 10). Pain intensity is equal in DM1, DM2 and other muscular dystrophies, and as severe as in patients with low back pain and osteoarthritis (5). Frequency of patients not using analgesics was about 50%, which is in accordance with previous studies (4, 6, 9). This might be due to the fact that pain response to analgesics therapy is quite poor in myotonic dystrophies (16). In our patients and in previously investigated DM2 cohort, analgesics reduced pain in about 65% of cases (7, 9). It is of mention that our

Table 4. Association of average pain intensity with investigated factors in patients with DM1 and DM2 (second linear regression).

Feature	DM1 (beta, p)	DM2 (beta, p)
Female gender	n.i.	+0.37, 0.005
Duration	+0.35, 0.047	n.i.
INQoL weakness	n.i.	+0.30, 0.060
INQoL emotions	n.i.	+0.64, 0.000
FSS	0.23, 0.203	n.i.
MMSE	-0.46, 0.011	n.i.
TMT-A	n.i.	+0.22, 0.093
INQoL relationships	n.i.	-0.04, 0.875
INQoL independence	+0.15, 0.384	+0.07, 0.665
R^2 adjusted	0.28	0.48

n.s. non-significant; n.i. not included in the second multivariate analysis since it was non-significant in the first multivariate analysis

patients used nonsteoroid antiinflammatory drugs and only a minority of them tried different options such as neuropathic pain medication. Physicians should be cautious with analgesics since they may interfere with multisystemic aspects of myotonic dystrophies.

In this study we identified different parameters associated with pain in myotonic dystrophies, opening new opportunities for pain therapy in DM1 and DM2. Although we performed a comprehensive analysis of different factors, they described only 28% of pain severity in DM1 and 50% in DM2. Therefore further studies are requested to find other significant dimensions of pain in order to improve the pain management in these diseases.

In DM1 the more severe pain was in strong association with a longer duration of disease and worse cognition measured with MMSE, in contrast with two previous papers that found no correlation between disease duration and pain severity in these patients. The authors hypothesize that patients in this study may have displayed adequate coping behavior to lessen pain severity (5, 6). However, patients with longer duration of DM1 had also lower quality of life (13, 29) which is in favor of inadequate coping mechanisms and maladjustments to the disease, probably due to the well-known brain impairment (30). In accordance with this, we found a strong correlation between pain severity and poorer achievement on MMSE. There is a possibility that subjects with cognitive impairment are not able to adequately process pain with impaired central nervous system. Also, Hosoi et al. (31) reported that patients with neuromuscular disorders sometimes are not able to express their emotional problems due to alexithymia, thus reporting more somatic complaints including pain. However, this relation might be bidirectional, i.e. pain might disturb patients during testing (6), though the latter is less possible since severity of pain was only mild to moderate. Our results suggest that cognitive behavioral therapy on people with DM1 might have positive impact on pain relief. One such multi-national trial named OPTIMISTIC is in progress in Europe (*http://www.muscular-dystrophy.org/research/news/7711_optimistic_trial_for_myotonic_dystrophy_type_1_launches_in_newcastle*).

In our DM2 cohort a strong association of pain severity with female gender and emotions was established. Female patients with neuromuscular disorders including DM1 reported more pain than males (1, 5). Since pain may be the first and the most disabling symptom of DM2, this might explain the fact that women usually predominate in series of DM2 patients. Depression is more common in DM2 patients with pain (7, 9). It is possible that DM2 patients are more depressed because of somatic pain, but also that pain is more commonly reported in patients with depression. Furthermore, patients with neuromuscular disorders and alexithymia probably report more pain (31). It seems possible that therapy for depression might also have positive impact on pain in DM2 patients.

Although DM2 seems to be less severe disease than DM1, QoL is equally impaired in both diseases, which is in accordance with a previous study (10). In our study the severity of pain correlated with QoL in both types of myotonic dystrophy. Suokas et al (10) and Udd et al. (7) observed lower QoL in DM2 subjects with pain, and several previous studies reported correlations of pain and QoL in DM1 (5). Tieleman et al. (10) found association between pain and QoL only in DM2, not in DM1 subjects.

This study has a few limitations: 1. Though myotonic dystrophies are rare diseases, to make stronger conclusions a larger number of patients should be necessary; 2. the lack of a control group from general population not permitting us to compare pain intensity and to conclude what pain features are disease-specific, and 3. the cross

sectional design. Follow-up studies are needed to resolve exact direction of observed correlations.

Conclusions

The majority of DM1 and DM2 patients have mild to moderate pain. These results open new opportunities for behavioral and cognitive interventions.

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Muscle histology changes after short term vibration training in healthy controls

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In search for additional counter measures of muscle atrophy vibration exercise training may have substantial effort for patients with neuromuscular disorders. To cover safety aspects and obtain muscle morphology data, a pilot study was performed in eleven healthy men. Countermovement jump, squat jump, drop jump and one repetition maximum test (1RM) were performed on a force platform before and after a 6 week training period. No severe side effects were found. Repeated needle muscle biopsies of the vastus lateralis muscle revealed a selective pre- to post-training type-2 myofiber hypertrophy of up to 50 %. The hypertrophy factors were 160 and 310, for type-2 myofibers. The mechanography system showed a significant increase in the 1RM maximum weight lifted (pre: 111,8 kg ± 11,5; post: 140,9 kg ± 13,00; p < 0,001). Vibration exercise is a safe and effective technique which desires further approval as counter measure in different types of neuromuscular atrophy.

Key words: muscle atrophy, muscle biopsy, muscle morphometry, vibration exercise

Introduction

Vibration stimulation exercise is a valuable training technique for athletes and counter measurement for any type of induced muscle atrophy. Vibration training is widely used as one of the prevention strategies of muscle aging. Only limited data are available about vibration induced effects on morphology of normal human skeletal muscle. Vibration applied to muscle or tendon induces a non-voluntary muscular contraction termed the "tonic vibration reflex" (1). Combined with substantial voluntary effort it was shown to elicit movements in neuromuscular patients who were unable to contract their paretic muscles (2). Vibratory stimulation of the muscle tendon evokes an excitation of muscle spindles mediated by 1a afferents and alpha-motor neurons. Additionally, it is suggested that central motor neuron control organization is activated (3, 4). Vibration stimulation of muscle may therefore enhance contraction and post-stimulation facilitation. Subsequently, vibration stimulated training may have positive effects in counter measures of muscle atrophy in spaceflight and disease related muscle atrophy. The aim of this pilot study was to establish morphological and morphometric data on vibration related effects in human muscle in healthy controls.

Probands and methods

The study was approved by the local Institutional Review Board of the University of Munich (vote 103/04). All participants gave written informed consent before they were included in the study.

Probands

11 healthy, non-elite sportsmen, mostly sport students (mean ± SD age, 26 ± 8.0 years, mean ± SD height, 1.83 m ± 0.06 m, mean ± SD weight; 80.3 ± 3.2 kg) were investigated. All probands had repeated needle muscle biopsies of the lateral vastus muscle before and after a 6-week course of high-frequency vibration training on a vibration platform (Galileo, Novotec medical, Germany). Testing was performed before and after the training intervention jumping power and force were measured by performing a countermovement jump (CMJ), a squat jump (SJ) and a drop jump (DJ), according to Schmidtbleicher (5), on a ground reaction force platform (Leonardo, Novotec Medical, Pforzheim, Germany), and a one repetition maximum test (1RM) was performed. The knee ankle during the 1RM squat set at 90° to be accepted. After each successful attempt the load was increased until failure in lifting the load. The probands followed a

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minimum of two minutes rest between the attempts in the 1RM. The pre test was performed at least two days after the biopsy and the post test at least two days after the last training.

Training

The 6-week training period consisted of a one week familiarisation period and 5 weeks of training with additional weight, starting with 40% of the body weight. Workouts with 2 sets of squats and a five minute warm up before the workout were performed twice a week. The participants trained in every set until exhaustion, with a two minute rest between the sets. The athletes had to be exhausted after a maximum of three minutes; therefore the weight was adapted to the progress. The weight was carried around the hip, with a special center - of mass dumb-bell. The participants performed the squats on the vibration device with 25 Hz. The position from the foot varied between the athletes (15-21 cm with the bunion from the center of the platform), according to the body proportions. Due to the construction (seesaw) of the vibration device, the amplitudes varied according to the foot position between a minimum of 2.9-3.9 mm (5.8-7.8 mm peak to peak).

Muscle biopsy specimens

Twenty-two needle samples were taken from the left vastus lateralis muscle. The first and second biopsy was done within the same area of about a 5-10 cm distance of the vastus lateralis muscle in all probands. All muscle specimens were processed using standard histological procedures. After biopsy, all parts were frozen in liquid nitrogen. Cryosections (8 µm) were routinely stained, including haematoxylin & eosin, reduced nicotinamide adenine dinucleotide-tetrazolium reductase (NADH), adenosine triphosphatase reactions (ATPase) at pH 4.6 and pH 10.4, modified Gomori trichrome, van Gieson, cytrochrome C oxidase, succinic dehydrogenase, Sudan black B, acid phosphatase, and periodic acid-Schiff. All sections were evaluated semiquantitatively by light microscopy according to the techniques and methods given by Dubowitz (6).

Morphometry

Quantitative morphometry was done on 20 stained samples. In six consecutive microphotographs of stains for type1 or 2 using ATPase at pH 4.6 and 10.4, the diameters of atrophic and hypertrophic myofibers (normal control range 40-80 µm for males) were measured. The number of muscle fibers evaluated in each sample ranged from 60 to 212. The hypertrophy factors were calculated according to Brooke and Engel using an imaging software (UTHSCSA ImageTool, alpha3, version 2c; San Antonio, Texas) (7, 8). Upper limits for the hypertrophy factor for normal quadriceps muscle are 150 for type 1 myofibers and 400 for type 2 myofibers for males (6-8).

Statistical analysis

All values for the performance tests and for the training in the text and tables are given in mean ± SD. Wilcoxon test with Monte Carlo significance was used for the comparison between pre and post test results. Significance was accepted at $P < 0.05$.

Results

Side effects

The repeated needle muscle biopsies of the lateral vastus muscle were without any notable side effects for the probands. Vibration stimulation exercise was very well tolerated by all participants. No adverse events directly related to the vibration were recordable.

Training results

The average training time on the vibration platform was 150 seconds per set, with an average of 63 squats until exhaustion. The participants started with an additional weight of 30.5 ± 2.8 kg, which conformed to 40 % of the body weight and increased the weight to 65.5 ± 8.2 kg in their last workout. Due to personal reasons two probands performed no training in the last week.

Test results

All participants performed pre and post testing on the force platform and the 1RM test. There were no significant changes between pre and post testing on the force plate (Fig. 1A). Only the weight lifted in the 1RM test did increase significantly (pre: 111.8 ± 11.5, post: 140.9 ± 13.0; $p < 0.001$) (Fig.1B).

Muscle biopsy

Nineteen out of 22 performed muscle specimen were included for the final analysis. Three needle biopsies showed mainly connective tissue and only a few muscle fibers, thus they were therefore excluded. Routine histology and histochemistry revealed complete normal muscle biopsy finding in all analysed specimen. Histochemistry did not show significant post-training alterations (e.g. subsarcolemmal mitochondrial increase). Before training, the hypertrophy factor of type 1 myofibers was 120, and 160 for type 2 myofibers respectively. After a 6-week high-frequency vibration stimulation exercises (frequen-

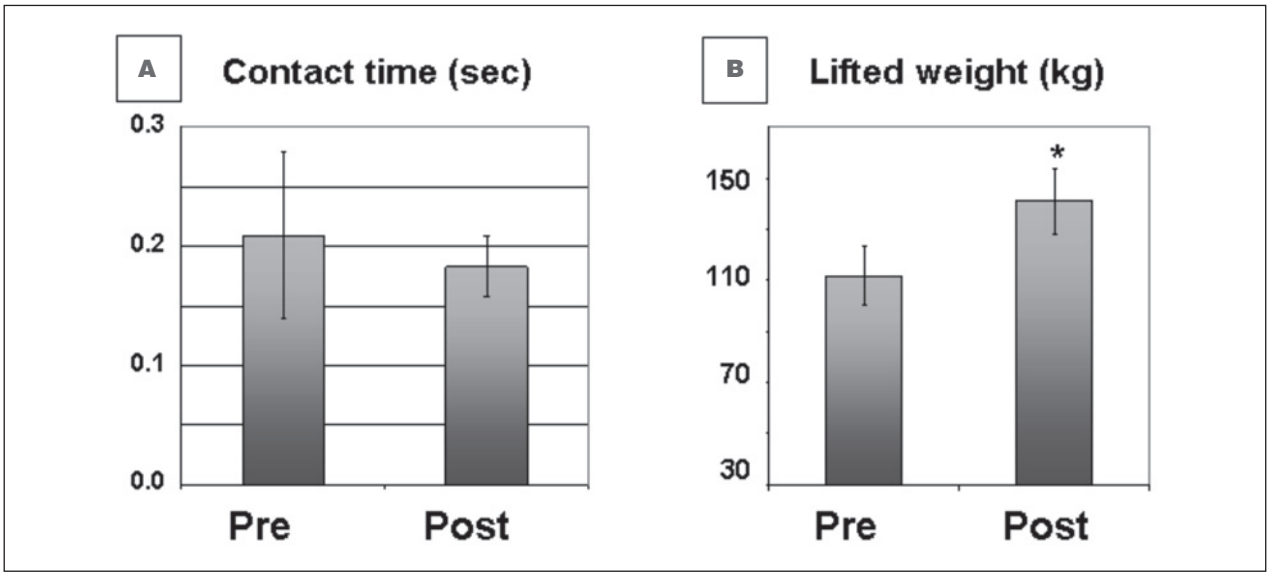


Figure 1. Training effects in vibration stimulated muscles. **A:** Non-significant reduction in measured contact time in the drop jump test (Pre 0.209 ± 0.07 sec.; Post 0.183 ± 0.025; $P = 0.06$). **B:** Significant increase in the lifted weight (kg) in the 1RM test (Pre 111.8 ± 11.5; Post 140.9 ± 13.0; $P < 0.001$).

cy 25 Hz, minimum amplitudes 2.9-3.9 mm), the type 1 fiber hypertrophy factor was 150, and the type 2 fiber hypertrophy factor was 310 (Figs. 2, 3).

Discussion

This study evaluates the influence of high-frequency vibration stimulation exercise training in healthy probands on muscle fiber type hypertrophy controlled by morphometrically analyzed repeated muscle biopsies. Compared to given values of normal controls, an statistically non-significant myofibers type related muscle hypertrophy was found (6-8). However, this study revealed, that high-frequency vibration training as short as 2 sets with a maximum of 3 minutes 2-times a week, induced a type-2 myofiber hypertrophy with an increase of up to 50% of the total number of hypertrophied type-2 myofibers beyond 80µm in diameter. Significant changes in three standard jumping tests were not found, although there are tendencies towards a reduction of contact time in the DJ. This increase could be related to an improvement in neuromuscular performance, documented in acute and chronic enhancements in strength and power (4, 9-11). The significant change in the 1RM test may be mainly related to the squats performed on the platform, but also strengthened by the vibration applied to the body. Campos et. al found that three different exercise protocols accomplished for 8 weeks (low 3-5 repetitions, intermediate 9-11 repetitions; high repetitions 20-28 repetitions) resulted all in an increase of the dynamic strength in the squat test. Their results showed, that the lower repetition group with more load improved the most. Although all three groups improved in the strength tests, significant changes in the fiber types were only found in the low and intermediate repetition groups with more resistance (12). Contrary to this, our study used a training protocol which, concerning the squat exercise, might only increase the muscular endurance according to other reports (13, 14). If any changes in muscular composition would occur, the changes could be more likely related to the vibration applied to the body. The participants in this study also trained with a shorter duration (6 weeks) and with less workouts (only twice a week). The 26 % increase in the 1 RM Test is comparable to the reported increase of 30% (12). Although vibration is known to improve jumping performance (11), the present study showed only tendencies towards a change. This might be related to a relatively short training time, which did not result in a significant improvement of neuromuscular performance. Finally, the optimal frequency of the vibration platform is still under debate. There is evidence that high-frequencies (> 50 Hz) might evoke a proportional increase in muscle tension, but this is almost absorbed by soft tissue (4). Additionally, increasing vibration frequency induced an increasing co-activation of antagonistic muscles (15). In contrast to this low-frequencies (<20 Hz) propagate through the kinetic chain to proximal muscles and may be absorbed by the human body tissue (4). Finally, we hypothesize, that different frequencies may stimulate distinct muscle fiber types in different muscles, which has

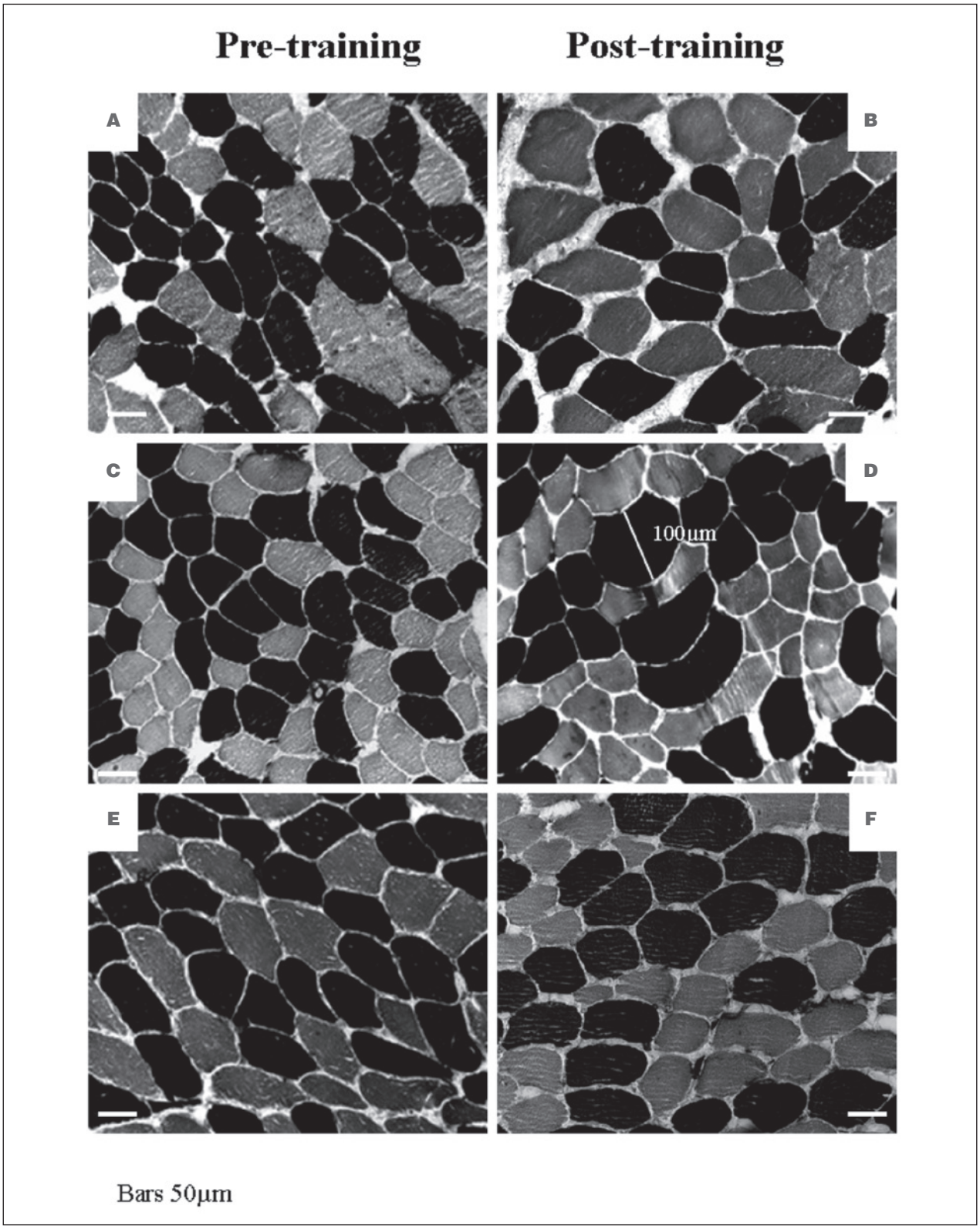
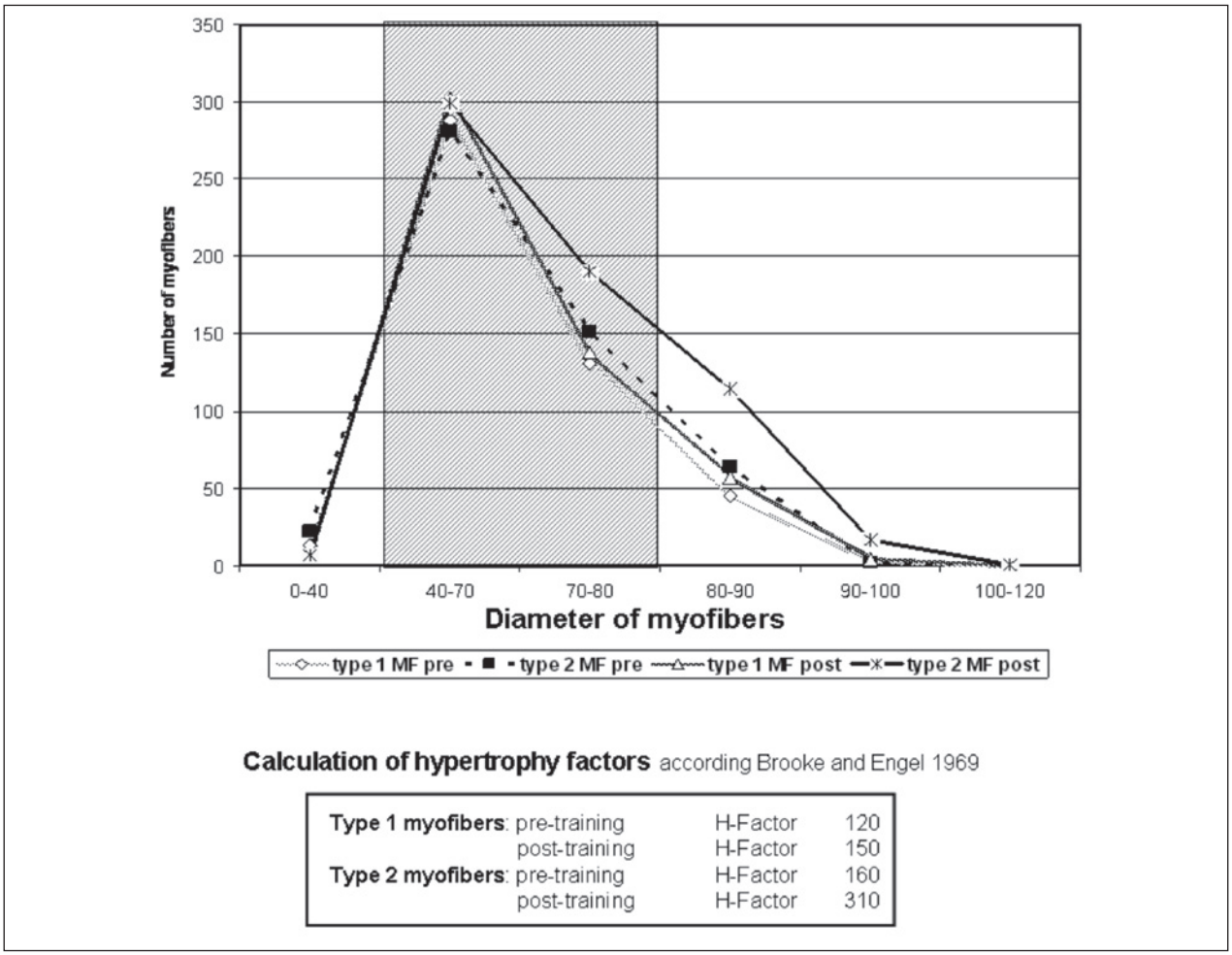


Figure 2. Pre and Post vibration training muscle morphology in 3 healthy sportsmen. Vastus lateralis muscle biopsy samples (ATPas histochemistry at pH 10.4) of three probands pre (**A, C, E**) and post (**B, D, F**) 6-week vibration platform training. **B, D, F** reveals an significant increase in type-2 myofiber diameters up to 100µm (**D**). Bars in A,B,C,E and F = 50µm.



Calculation of hypertrophy factors according Brooke and Engel 1969

Type 1 myofibers:	pre-training	H-Factor	120
	post-training	H-Factor	150
Type 2 myofibers:	pre-training	H-Factor	160
	post-training	H-Factor	310

Figure 3. Morphometric histogram and hypertrophy factor calculation (details see text).

to be further analysed. In this study we showed, that a 25 Hz vibration stimulus induced a type-2 myofiber hypertrophy in human vastus lateralis muscle. In order to improve neuromuscular performances we believe that different frequencies (slow versus fast) within a training period have to be tested. Moreover, this protocol has to be adapted to distinct types of muscle atrophy with a more proximal or distal pronounced weakness.

In summary, vibration stimulation exercise is a safe and effective technique which may help to improve neuromuscular performance in health, mitigate spaceflight muscle atrophy, and muscle atrophy associated with neuromuscular disorders and muscle ageing.

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CASE REPORT

Rhabdomyolysis in hyponatremia and paraneoplastic syndrome of inappropriate antidiuresis

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We report a 26-year-old woman admitted to our hospital for generalized tonic seizure. Laboratory investigations revealed severe hyponatremia possibly triggered by vomiting and diarrhea. 24 hours after correction of hyponatremia she developed diffuse myalgias and marked hyperCKemia. Syndrome of inappropriate antidiuresis (SIAD) was suspected as cause of hyponatremia. Abnormal vaginal bleeding prompts gynecological evaluation and a small-cell carcinoma of uterine cervix was detected.

Key words: rhabdomyolisis, hyponatriemia, syndrome of inappropriate antidiuresis, small cell neuroendocrine carcinoma

Case report

A 26-year-old woman was admitted to our hospital after generalized tonic seizure. There was no family history of epilepsy, psychiatric disease, neuropathy and myopathy. She smoked 10 cigarettes a day and was taking oral contraceptive therapy. No other medications, alcohol consume, drug abuse or allergies were reported nor trauma. Medical history was irrelevant, physical and neurological examination were normal. At the admission, laboratory investigations revealed severe euvolemic hypotonic hyponatremia (107 mEq/l) possibly triggered by vomiting and diarrhea occurred in the previous 3 days. Twenty-four hours after the correction of hyponatremia by the intravenous administration of normal saline solution (NaCl 0.9% saline) she developed diffuse myalgias associated with laboratory evidence of marked elevation of creatine kinase (CK) level (Table 1). There was no evidence of muscle trauma, stiffness or swelling and a preserved renal function and diuresis were observed throughout the evolution. An extensive diagnostic workup (Table 2) excluded other presumed causes for rhabdomyolysis, so a diagnosis of rhabdomyolysis secondary to hyponatremia and/or its correction was made. In particular a diagnosis of *Syndrome of inappropriate antidiuresis* (SIAD) was suspected as the cause of euvolemic hypotonic hyponatremia as confirmed by diagnostic criteria of decreased serum osmolality (225mOsm/kg) and elevated urine osmolality (475 mOsm/kg) in the absence of renal, adrenal and thyroid insufficiency. Oral fluid restriction (1.5 lt/day) and salt tabs supplementation (200 mEq/day) maintained serum sodium level in a non-critical range (122 mEq/l). Abnormal vaginal bleeding prompted a gynecological evaluation that revealed a small-cell carcinoma of uterine cervix. Surgical treatment followed by chemoteraphy and radiotherapy resulted in the resolution of paraneoplastic SIAD and normalization of hyponatremia.

Discussion

Seizure and rhabdomyolysis are uncommon serious complications of severe acute hyponatremia and / or its correction (1, 2). Rhabdomyolisis is a potentially life-threatening syndrome resulting from lyisis of skeletal muscle fibres with release of intracellular product into systemic circulation (3). It may be due to failure in cell volume regulation and ionic balance ultimately affecting membrane homeostasis and cell integrity (4). Syndrome of inappropriate antidiuresis (SIAD) is a disorder

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Table 1. Laboratory data trends.

Time(h)	Na (mEq/l) (134-146)	K (mEq/l) (3.6-5.4)	CK (IU/l) (90-205)	AST (IU/l) (5-37)	ALT (IU/l) (5-40)	LDH (IU/l) (125-243)	crea (mg/dl) (0.4-1.2)	POsm (mOsm/kg) (280-300)	UOsm (mOsm/kg)	UNa (mEq/24h) (50-200)
Admission (0)	107	3.9		37	22	274	0.69	225		
2	108									
28	122	3.9	26535	168	42	772	0.70	255	475	258
100	119		39561	476	372					
124	113		22508	157	166					
148	112									
194	110		1653							
Dimission	114	4.5	314							

Table 2. Laboratory and instrumental investigations.

Standard hematological and byochemisty:	Normal
fT3-fT4-TSH, ACTH, cortisolemia, cortosoluria/24 h:	Normal
Clino/orthostatism plasma renin activity:	Low
Aldoserone:	Normal
Neoplastic markers:	Negative
Infections (VDRL, HBV, HCV, EBV, CMV, HSV1-2, VZV abs):	Negative
Stool colture: negative for Salmonelle, Shigelle, Campylobacter Rotavirus, Adenovirus, Norovirus Ag:	Negative
ECG:	Normal
EEG:	Normal
Brain CT and MRI:	Normal
EMG/ENG:	Mild myopathic pattern
Chest XR:	Normal
Abdomen/pelvic echography:	Normal

of sodium and water balance and is a major cause of euvolemic hypotonic hyponatremia (5). Ectopic production of antidiuretic hormone (ADH) by tumor, mainly small-cell neuroendocrine carcinoma (SNEC), is one of the most common causes of SIAD (6-8) and is exceptionally described in small-cell carcinoma of uterine cervix (9). We describe the case of a patient with a small-cell neuroendocrine carcinoma of uterine cervix presenting with generalized seizure and rhabdomyolysis related to severe hyponatremia, secondary to paraneoplastic SIAD. The case here reported suggests that an aggressive treatment to correct hyponatremia should be avoided. Furthermore, a careful monitoring for rhabdomyolysis is necessary to prevent and treat the possible complications. Paraneoplastic SIAD is one of the most common cause of euvolemic hypotonic hyponatremia and should be thoroughly investigated in particular for small-cell neuroendocrine carcinoma often difficult to detect. Small cell carcinoma of the uterine cervix is a rare variant of SNEC taking up only 0.5% to 5% of the type of cervical cancer and is rarely associated with SIAD as in our case (9). Extensive evaluation of SIAD has great implication on the diagnosis, treatment, follow-up and prognosis of this extremely aggressive tumor.

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PROCEEDINGS FROM MEETINGS

Minutes of the European POmpe Consortium (EPOC) Meeting

March 27 to 28, 2015, Munich, Germany

Benedikt Schoser[1], Pascal Laforêt[2], Michelle E. Kruijshaar[3], Antonio Toscano[4], Pieter A. van Doorn[5] and Ans T. van der Ploeg[3], on behalf of the European POmpe Consortium (EPOC)

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In September 2014, twenty-two experts in the field of Pompe disease from nine European countries assembled in Naarden, The Netherlands, for a workshop on Pompe disease, the 208th ENMC International Workshop "Formation of a European Network to develop a European data sharing model and treatment guidelines for Pompe disease" (1). Here, the European POmpe Consortium (EPOC) was funded. To continue our work, the consortium met for the second time, March 27 to 28, 2015 in Munich, Germany.

Friday March 27, 2015
Introduction and summary

The meeting was opened by Benedikt Schoser who welcomed all attendees to Munich and summarized the agenda.

Ans van der Ploeg gave an overview of the first meeting of the consortium in September 2014 in Naarden. At this first meeting the EPOC consortium was founded, a minimal dataset was agreed for adult patients, as well as criteria for starting and stopping enzyme replacement therapy (ERT) in adult patients. She elaborated shortly on the scope of the consortium: i.e. via concerted actions of the partners we aim to i) improve prospects for patients by combining efforts on understanding the disease process, existing therapies and development of innovative treatment strategies; ii) to provide guidance on treatment, care and outcome measures, iii) to harmonize the views on access and reimbursement of therapies in Europe in order to create equal chances for patients with Pompe disease across Europe.

After the first meeting in Naarden, the steering group for the Naarden and Munich meetings proposed the name European POmpe Consortium (EPOC). All attendees agreed to adopt this name.

Michelle Kruijshaar presented the progress on publications resulting from the Naarden meeting. A lay summary was posted on the ENMC website in September 2014. The full workshop report is now available via the Journal "Neuromuscular disorders" (1). Finally, a separate manuscript is being prepared to describe criteria for starting and stopping ERT in adult Pompe patients. The proposed start and stop criteria for adult patients were reviewed. A few remarks from the group were made regarding the phrasing of certain stop criteria, which will be incorporated in the manuscript that is being prepared.

Consortium agreement

Benedikt Schoser presented a proposal of the steering group for the organizational structure of the consortium.

Address for correspondence: Benedikt Schoser, Friedrich-Baur-Institute, Dept. of Neurology, Ludwig-Maximilians-University, Ziemssenstr. 1a, 80336 Munich, Germany. E-mail: Benedikt.Schoser@med.uni-muenchen.de@med.uni-muenchen.de

This was discussed in detail. The following was agreed:

1. General Assembly (GA):
   a. Is comprised of all approved members of the consortium.
   b. Meets at least every one or two years.
   c. At the meeting, members can vote board members and approve new members of the consortium.
2. Members:
   a. Have to be professionals (clinicians and scientists) with experience on Pompe disease working in Europe (or Switzerland).
   b. Have voting rights.
   c. One IPA representative shall be a member, i.e. with voting rights.
3. Board:
   a. Acts for the GA.
   b. Is comprised of 5 members: a chair, a co-chair, a secretary/treasurer, and two general board members.
   c. Board members are elected by the GA. Every 2 years, 2 board members shall be substituted. It is possible to be re-elected once.
   d. Proposals for new members of the Consortium will be put to the board before being agendized for the GA meeting.

Next, an election was held for the first steering board by secret ballot (i.e. on paper not by raising hands). The first elected board is:

- Benedikt Schoser (chair)
- Ans van der Ploeg (co-chair)
- Pascal Laforêt (secretary)
- Antonio Toscano
- Pieter van Doorn

Finally, a discussion followed on the need for, and content of, a consortium agreement and statutes for the consortium. Example documents were circulated in advance of the meeting. It was agreed that a consortium agreement should be made first; then the EPOC statute should be provided. The content should be similar to the elements included in the example document, specifying the organizational structure of the consortium, and specifying that at present there is no fee attached to membership.

How to collect data

To further our understanding of the disease processes and existing therapies the consortium wants to/ will collect data from the different countries. Antonio Toscano and Pieter van Doorn chaired the session on how data could be organized. They presented a number of decisions that have to be made when considering data sharing. The first point, whether or not there should be a central database, received a lot of discussion. Sabrina Sacconi expressed concern that data would be collected without sufficiently important research questions driving this data collection. Other members from Italy and France supported the need for data collection to be driven by specific research questions. Another point that was made was the importance of having a clear publication policy that includes all centers that provide data. Last, it appeared that rules regarding sharing data and requirements for ethics differ between countries. As next steps it was decided to first focus on the research questions ("in a project by project" way), and that an inventory of the rules and requirements for the different countries should be made.

National presentations and scientific ideas

Pascal Laforet and Antonio Toscano chaired the session on national presentations and scientific ideas. Presentations by the individual countries (of about 5 minutes) were given, including the total number of patients, number of centers and distance to the centers, as well as the main clinical and scientific interests in these countries.

Adding up the entire patient numbers of the 9 countries (Italy, Spain, France, the Netherlands, the UK, Denmark, Switzerland, Austria and Germany), our consortium will cover at least 1250 Pompe patients of all ages. This gives a prevalence of Pompe disease of about 1 in 283,000 (1250 / 354 million inhabitants; given that all the patients included in the presentations are still alive).

Presentations from the UK and the Netherlands indicated that infusions of myozyme are commonly done in the home setting. Other countries were interested in the experience with home infusions and it was suggested that it would be helpful if these experiences were published.

Saturday March 28, 2015 Discussion of possible grant proposals

Ans van der Ploeg informed all participants of an E-Rare proposal that has been sent out. E-Rare aims to stimulate international, interdisciplinary projects. There are several limitations to the proposal, including which countries are eligible for participating. A proposal was sent in under the lead of Ans van der Ploeg, supported by Pascal Laforet, Benedikt Schoser, Antonio Toscano, and Beril Talim (all E-Rare eligible countries) for a project called PrEDIcTION (Pompe disease EDiting by Treatment OptimizatION). The proposal has successfully passed the first evaluation round, and is presently in the second round of the E-Rare evaluation process.

Other calls that may be interesting for the consortium include: i) COST program of EU (funds meetings); ii) apply for an AMDA grant for a specific EPOC project, iii) German organization for rare disease database (model registry).

Progress on workgroups

Andreas Hahn and Alexander Broomfield presented the progress of the infant workgroup on behalf of the four countries (Germany, UK, Italy and The Netherlands). The workgroup has chosen to focus on a specific research question concerning the dosing regimens that are applied in Europe. Data from two of the four participating countries has already partially been collated and all four countries have already made some effort to collate their national data. A total of around 130 infant patients are expected to be available after combining these results. The preliminary data show that different dosing regimens are applied in Europe and gave a first impression what can be done with the concerted action of the consortium.

Nadine van der Beek presented the progress on the R-PACT project. A traditional assessment scale does not always have interval properties (i.e. the difference between response options is not necessarily the same and/or a sum score of 3 may identify people with different clinical problems). The Rash method can help to develop scales with interval properties. In Rotterdam, a scale to measure activity and participation of Pompe patients was developed using this methodology. In the original study it was developed and validated for the Dutch and English language. For the present project it has been translated into French, German and Italian, and other translations can be made. Pascal Laforet, Eugen Mengel and Jordi Díaz Manera have indicated that they are interested to participate in this project.

Nadine van der Beek also briefly presented the Rasch-built MRC score. This has only 4 levels, compared to the traditional MRC score which has 6 levels. Some centres may be interested to assess both MRC scores, but it was decided that at least the traditional MRC score remains part of the minimal dataset.

Next steps

The next steps of the network will be to discuss which specific research projects the network will focus on and how data can be shared for these projects. The consensus on criteria for starting and stopping ERT in adult patients will be harmonized and published. Finally, outcome measures and start and stop criteria for infants and children will be discussed.

Next meeting

It was proposed that a meeting of about 3 hours would be held at the World Muscle Conference in Brighton (September 30, 2015). The steering board will send out a meeting invitation and organize a meeting room in Brighton. Pascal Laforet offered Paris as the location for the next EPOC meeting in 2016.

List of participants (European Pompe Consortium, EPOC, members):

France: Pascal Laforêt *(Paris)*, Claude Desnuelle *(Nice)*, Sabrina Sacconi *(Nice)*
Spain: Ignacio Pascual Pascual *(Madrid),* Jordi Díaz Manera *(Barcelona)*
Italy: Antonio Toscano *(Messina),* Tiziana Mongini *(Turin),* Corrado Angelini *(Venice),* Giancarlo Parenti *(Naples)*
UK: Mark Roberts *(Manchester),* Alexander Broomfield *(Manchester)*
Switzerland: Kai Rösler *(Bern),* Oliver Findling *(Aarau)*
Germany: Andreas Hahn *(Giessen),* Eugen Mengel *(Mainz),* Benedikt Schoser *(Munich),* Wolfgang Müller-Felber *(Munich),* Angela Schüller *(Munich),* Federica Montagnese *(Munich/Messina),* Stephan Wenninger *(Munich)*
Netherlands: Ans van der Ploeg *(Rotterdam)* Nadine van der Beek *(Rotterdam),* Pieter van Doorn *(Rotterdam),* Michelle Kruijshaar *(Rotterdam),* Pim Pijnappel *(Rotterdam)*

Members with cancellation for the Munich meeting:
Denmark: John Vissing / Nicolai Preisler *(Copenhagen)*
Turkey: Beril Talim *(Ankara)*
Austria: Christian Eggers *(Linz),* Thomas Stulnig *(Vienna)*
UK: Ashok Vellodi, Robin Lachmann, Ros Quinlivan *(London)*
Belgium: Peter van den Bergh *(Brussels)*
Switzerland: Thomas Hundsberger *(St. Gallen),* Marianne Rohrbach *(Zurich)*
Germany: Ursula Plöckinger *(Berlin),* Peter Young, Matthias Boentert *(Münster),* Rudolf Kley *(Bochum),* Cornelia Kornblum *(Bonn),* Julia Hennermann *(Mainz)*
Patient representative (IPA): Thomas Schaller *(Weingarten)*

References

1. Schoser B, Laforêt P, Kruijshaar ME, et al. 208th ENMC International Workshop: Formation of a European Network to develop a European data sharing model and treatment guidelines for Pompe disease Naarden, The Netherlands, 26-28 September 2014. Neuromuscul Disord 2015;25:674-8.

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OBITUARY

Professor Irena Hausmanowa-Petrusewicz (1917-2015)

"The End is as invisible as the Beginning"*

In the early morning of 7th July 2015, the Secretary of the Neuromuscular Unit, Mrs Ewa Witkowska, received a call from a daughter of our Professor – Prof. Marta Petrusewicz, informing her of the death of her mother.

For a few months in the empty room of the Head of the Warsaw Neuromuscular Unit, the box fixed on the 7th July on the calendar remained immobile and unchanged.

Now, seven months after the death of Prof. Hausmanowa, when her room has at last been reorganised and refurnished, the feeling of emptiness grows with each passing day.

The small gallery of portraits of the Prof. Hausmanowa's friends and teachers has now been transferred to the Lecture Hall of our Institute.

Every morning, also including the dark and cold winter ones, Prof. Hausmanowa was in her room at 7.00 a.m. She liked very much these times first thing mornings without the ringing phone, when she could work and contemplate in the silence. Then – when – the phones did indeed started to ring, the Professor deployed that special patience and kindness of hers to talk with the mothers of sick children. And some of them were inclined to call very regularly.

Every Tuesday, the Professor would run consultations with the patients coming to the Neuromuscular Unit from all parts of Poland. It was an honour for us working at the Unit to see and be part of this, and a special lesson in medicine to learn how Professor Hausmanowa would deal with the sick, often having to communicate with them diagnosis of a severe disorder. From her we learnt the art of talking to patients. The patient was always the centre of attention for the Professor and the scientific issues were always closely linked with the clinical, which Professor Hausmanowa strived to understand and solve, using all available scientific methods.

For more than 60 years, Professor Hausmanowa developed the modern science of neuromuscular disorders in Poland, starting from morphology, through biochemistry, and electrophysiology, to genetics. Professor Hausmanowa possessed a special ability to connect narrow scientific disciplines, and deploy them correctly solving the problems of the particular patient.

The total sum of her papers covering a wide spectrum of the neurological sciences are brought together in no fewer than 13 volumes.

Publications she paid particular attention to were new editions of the *Polish Handbook on Neuromuscular Disorders* first coming out in 1967. And indeed, the new editions of *Choroby nerwowo-mięśniowe* under her editorship kept on appearing, every couple of years.

The Professor was a pioneer of clinical electrophysiology in Poland, and a handbook of hers dealing with this subject was also published. She was also a pioneer implementer of genetics in the neurological clinic. And every year thanks to Professor Hausmanowa, we were able to host the outstanding experts in the field of neuromuscular disorders. We thus, had direct access to the latest scientific achievements, and to their authors.

Professor Hausmanowa always discussed with us the plans we had for papers to be presented during neuromuscular conferences. Equally, the name-days of members of the team were never forgotten, and those that happened to fall in spring would be graced by sprigs of flowering lilac for the celebrants from the Professor's garden. Here also was a person of unusual self-deprecation and a very original sense of humour!

Just one month before death, she came to the Neuromuscular Unit for the last time to consult with the patients.

In 2004, at one of the aforementioned name-day gatherings, she presented one of us with the book *Roman Triptych*, as authored by His Holiness Pope John Paul II. And in the chapter Apocalypsis of the poetic *Roman Triptych* there is a sentence: "*And yet I do not altogether die, what is indestructible in me remains*"*.

Andrzej Kochański
Neuromuscular Unit
Mossakowski Medical Research Centre
Polish Academy of Sciences, Warszawa, Poland

* John Paul II: *Roman Triptych Meditations*, translated by Jerzy Peterkiewicz, Wydawnictwo Literackie Kraków 2003.

Prof. Irena Hausmanowa and her working group.

NEWS FROM AROUND THE WORLD

MSM

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

The symposium was in the traditional two-days MSM format with 5 selected topics:

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

During the General Assembly of the Society, it was decided to add to the members of the Board in office Haluk Topaloglu (Ankara, President elect), Kyproula Christodoulou (Cyprus, Vice President and Secretary), and Vincenzo Nigro (Coordinator of the Task Force for Genetics / NGS).

GCA

During the Gala dinner of the 12th Congress of the Mediterranean Society of Myology held in Naples, Italy on May 18th-20th, the 2015 Gaetano Conte Prizes were assigned to Haluk Topaloglu (Ankara) and Claude Desnuelle (Nice) ex aequo for clinical research (see photos).

AIM

During the recent AIM Congress held in Naples in May 2015, AIM has renewed its board for the next three years. President has been elected Prof. Gabriele Siciliano (Pisa), Secretary is Dr. Massimiliano Filosto (Brescia), Treasurer is Prof. Carmelo Rodolico (Messina).

Prof. Maurizio Moggio (Milan) as immediate past-President and the advisors Elena Pegoraro (Padua) Antonino Muzio (Chieti), Giovanni Antonini (Roma), Stefano Previtali (Milan), Marina Mora (Milan) and Chiara Fiorillo (Genoa) are also part of the Board.

AIM is now conducting new initiatives in the field of dissemination of knowledge on muscle diseases within the neurological scientific context, both at national and international levels, as well as in implementing the role of its associates in the fields of health assistance making decision and treatment delivery, network collaborations for disease registries and exchanges with other scientific communities to improve the level of the standards of care.

An update of the survey for Clinical Centres for Muscle Diseases is now running on to get a picture of the several activities performed at the various sites throughout the country, in terms of diagnosis, treatment, scientific research and relationships with patients' Organization.

On the AIM website (www.miologia.org) you may also consult recent guidelines in neuromuscular disease management and a list of upcoming events sponsored by the Association.

The next AIM Congress will be held in Lecce from 8 to 11 June 2016. The preliminary program and deadline for submission of abstracts and application are available on the website www.aim2016.it.

LGMD-EuroNet

On Friday 22nd May 2015 the LGMD EuroNET had its second meeting at the hotel "Royal Continental" in Naples, organized by GFB ONLUS during the 15th AIM Congress 2015. The topics for discussing were the following:

1. Request to ENMC to organize in 2016 an ENMC Workshop on Limb Girdle Muscular Dystrophies.
2. Participation of LGMD EuroNET to European Project (calls 2015 of Horizon 2020).
3. COST on Limb Girdle Muscular Dystrophies.

The following members of LGMD EuroNET participated to the meeting: Angelini Corrado (Venezia), Bruno Claudio (Genova), Comi Giacomo (Milano), Cudia Paola (Venezia),, D'angelo Maria Grazia (Lecco), Di Fruscio Giuseppina (Napoli), Gorni Ksenija (Milano) , Ionica Elena (Bucarest) , Minetti Carlo (Genova), Mora Marina (Milano), Nigro Vincenzo (Napoli), Sandona' Doriana (Padova), Savarese Marco (Napoli), Semplicini Claudio (Padova), Tasca Elisabetta (Venezia), Torrente Yvan (Milano), Vola Beatrice, President Gfb Onlus (Sondrio).

Other Participants: Azan Gaetano, Baratto Serena (For Elisabetta Gazzerro,Genova), Bello Luca (Padova), Giugliano Teresa, Passamano Luigia (For Politano Luisa, Napoli).

Chairman of the meeting was Prof. Angelini Corrado, secretary Vola Beatrice. Angelini open the discussion on the different topics.

1. Workshop ENMC on Limb Girdle Muscular Dystrophies.

Prof. Angelini Corrado is willing to apply the request at the European NeuroMuscular Centre (ENMC), to organize a Workshop on Limb Girdle Muscular Dystrophies titled "LGMD Boundaries, genetic tools and treatment guidelines". The other applicants (workshop organisers) will be Prof. John Vissing and Bruno Eymards.

Three will be the aims to this workshop:

- To define LGMD infantile-adult borders
- To define typical pathologies for LGMD
- To analyse new diagnostic tools, such as NGS, and to define trial readiness.

Primary outcomes will be:

- To collect experience from different countries and analyse the genetic tools and biomarkers.
- To assess the feasibility of trials and to delineate possible outcomes.

The assembly will prepare the list of potential participants to the workshop.

2. Participation to the European Projects of LGMD Euronet (Calls 2015 of Horizon 2020).

LGMD EuroNET is mainly focusing on the topics 2 and 7, similar to the previous work programme:

- Topic 2 - Diagnostic characterisation of rare diseases , for which is available an estimated budget of 10-12 milion of euros, that implies a very large partnership, also considered the proposed challenge.
- Topic 7 - New therapies for rare diseases, that will be open only at the end of 2016, expiring in 2017.

The assembly discussed a possible project for the topic 7, i.e. to develop, thanks to international doctors involved, a solid international consortium, COST.

Beatrice Vola presented her ideas on the topic 7 illustrating the projects of gene therapy in LGMD2D and LGMD2E recently developed in Columbus (USA) by Prof. Jerry Mendell.

3. Request for a COST (European Cooperation in Science and Technology) foundation for Limb Girdle Muscular Dystrophies

The Assembly decides to proceed with the request of the European Cooperation in Science and Technology (COST) constitution in the field of Limb Girdle Muscular Dystrophies.Further information will be available on the website www.lgmd2e.org

WMS

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

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

The 21st International WMS Congress will be held in Granada (Spain) from 4 to 8 October 2016.

Gaetano Conte Prize assigned to Prof. Claude Desnuelle.

Gaetano Conte Prize assigned to Prof. Haluk Topaloglu.

FORTHCOMING MEETINGS

October 6-10

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

2016

February 29-March 02

6th World Congress on Cell & Stem Cell Research. Philadelphia, Pennsylvania, USA. Information: website: *http://stemcell.omicsgroup.com/*

March 7-9

NeurOmics Annual Meeting 2016. Barcellona, Spain. Information: website: *www.meeting.rd-neuromics.eu*

March 9-11

RD-Connect Annual Meeting 2016. Barcelona, Spain. Information: website: *www.meeting.rd-neuromics.eu*

March 14-18

5th International Congress of Myology. Lyon, France. Information: website: *www.myology2016.org*

March 17-20

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

April 3-7

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

May 19-21

5th International Conference and Exhibition on Cell and Gene Therapy. San Antonio, USA. Information: website: *http://cellgenetherapy.conferenceseries.com*

May 21-24

The European Human Genetics Conference. Barcelona, Spain. Information: website: *www.eshg.org*

May 25-27

International Cardiovascular Genetics Conference. Brisbane, Australia. Information: website: *www.iccgconference.com*

June 8-11

16th Congress of the Italian Association of Myology, Lecce, Italy. Information: website: *www.aim2016.it*

August 1-3

9th World Cardiology Congress. Manchester, UK. Information: *http://worldcardiology.conferenceseries.com*

September 2-6

45th European Muscle Conference. Montpellier, France. Information: website: *www.emc2016-montpellier.com*

September 4-9

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

September 12-13

2nd International Conference & Exhibition on Tissue preservation and Bio-banking. Philadelphia, USA. Information: website: *http://biobanking.conferenceseries.com/*

September 12-14

6th International Conference on Genomics & Pharmacogenomics. Berlin, Germany. Information: website: *http://genomics.conferenceseries.com/*

September 26-28

2nd International Conference and Exhibition on Molecular Medicine and Diagnostics. Orlando, USA Information: *website: http://molecularmedicine.conferenceseries.com/*

October 4-8

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

October 20-24

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

October 17-21

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

October 31- November 2

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

To be announced

22nd Congress of World Muscle Society. St. Malo, France. Information: website: *www.worldmusclesociety.org*

2018

October 16-20

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

To be announced

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

2019

October 22-26

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

To be announced

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

2020

October 27-31

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

To be announced

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

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For application or renewal to MSM

MEDITERRANEAN SOCIETY OF MYOLOGY* (MSM)

G. Nigro, President; H. Topaloglu, President Elected
L.T. Middleton, G. Siciliano, Vice-Presidents
K. Christodoulou, Secretary
L. Politano, Treasurer

APPLICATION/RENEWAL FORM

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

Prof. Luisa Politano, MSM Treasurer, Viale dei Pini 101, 80131, Napoli, Italy
Fax: 39 081 5665100 E-mail: actamyologica@gmail.com; luisa.politano@unina2.it
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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.

E-mail submission
Manuscripts will be sent to the Editorial Office *(actamyologica@gmail.com • luisa.politano@unina2.it)* by email only, with a covering note, subject to prior agreement from the Editorial Office, according to the following categories:
Original articles (maximum 5000 words, 8 figures or tables). A structured abstract of no more than 250 words should be included.
Reviews, Editorials (maximum 4000 words for Reviews and 1600 words for Editorials). These are usually commissioned by the Editors. Before spontaneously writing an Editorial or Review, it is advisable to contact the Editor to make sure that an article on the same or similar topic is not already in preparation.
Case Reports, Scientific Letters (maximum 1500 words, 10 references, 3 figures or tables, maximum 4 authors). A summary of 150 words may be included.
Letters to the Editor (maximum 600 words, 5 references). Letters commenting upon papers published in the journal during the previous year or concerning news in the myologic, cardio-myologic or neuro-myologic field, will be welcome. All Authors must sign the letter.
Rapid Reports (maximum 400 words, 5 references, 2 figures or tables). A letter should be included explaining why the author considers the paper justifies rapid processing.
Lectura. Invited formal discourse as a method of instruction. The structure will be suggested by the Editor.
Congress Proceedings either in the form of Selected Abstracts or Proceedings will be taken into consideration.
Information concerning new books, congresses and symposia, will be published if conforming to the policy of the Journal.
The manuscripts should be arranged as follows: 1) Title, authors, address institution, address for correspondence; 2) Repeat title, abstract, key words; 3) Text; 4) References; 5) Legends; 6) Figures or tables. Pages should be numbered (title page as page 1).
Title page. Check that it represents the content of the paper and is not misleading. Also suggest a short running title.
Key words. Supply up to three key words. Wherever possible, use terms from Index Medicus – Medical Subject Headings.
Text. Only international SI units and symbols must be used in the text. Tables and figures should be cited in numerical order as first mentioned in the text. Patients must be identified by numbers not initials.
Illustrations. Figures should be sent in .jpeg or .tiff format. Legends should be typed double-spaced and numbered with Arabic numerals corresponding to the illustrations. When symbols, arrows, numbers, or letters are used to identify parts of the illustrations, each should be explained clearly in the legend. For photomicrographs, the internal scale markers should be defined and the methods of staining should be given.
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Tables. Tables should be self-explanatory, double spaced on separate sheets with the table number and title above the table and explanatory notes below. Arabic numbers should be used for tables and correspond with the order in which the table is first mentioned in the text.
References. Reference numbers in the text must be in brackets. References in the list must be numbered as they appear in the text.
Standard journal article: Figarella-Branger D, Bartoli C, Civatte M, et al. Cytokines, chemokines and cell adhesion molecules in idiopathic inflammatory myopathies. Acta Myol 2000;19:207-8.
Books and other monographs: Dubowitz V. Muscle disorders in childhood. London: WB Saunders Company Ltd; 1978.
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