J Neurol (2012) 259:601–610
DOI 10.1007/s00415-011-6193-8

REVIEW

Muscle biopsy

G. Meola · E. Bugiardini · R. Cardani

Received: 1 July 2011 / Revised: 12 July 2011 / Accepted: 14 July 2011 / Published online: 30 July 2011

Abstract Muscle biopsy is required to provide a definitive diagnosis in many neuromuscular disorders. It can be performed through an open or needle technique under local anesthesia. The major limitations of the needle biopsy technique are the sample size, which is smaller than that obtained with open biopsy, and the impossibility of direct visualization of the sampling site. However, needle biopsy is a less invasive procedure than open biopsy and is particularly indicated for diagnosis of neuromuscular disease in infancy and childhood. The biopsied muscle should be one affected by the disease but not be too weak or too atrophic. Usually, in case of proximal muscle involvement, the quadriceps and the biceps are biopsied, while under suspicion of mitochondrial disorder, the deltoid is preferred. The samples must be immediately frozen or fixed after excision to prevent loss of enzymatic reactivity, DNA depletion or RNA degradation. A battery of stainings is performed on muscle sections from every frozen muscle biopsy arriving in the pathology laboratory. Histological, histochemical, and histoenzymatic stainings are performed to evaluate fiber atrophy, morphological, and structural changes and metabolic disorders. Moreover, immunohistochemistry and Western blotting analysis may be used for expression analysis of muscle proteins to obtain a specific diagnosis. There are myopathies that do not need muscle biopsy since a genetic test performed on a blood sample is enough for definitive diagnosis. Muscle biopsy is a useful technique which can make an enormous contribution in the field of neuromuscular disorders but should be considered and interpreted together with the patient's family and clinical history.

Keywords Muscle biopsy · Neuromuscular disorders · Muscular dystrophies · Inflammatory myopathies · Toxic and drug-induced myopathies

G. Meola (✉) · E. Bugiardini
Department of Neurology, IRCCS Policlinico San Donato, University of Milan, Via Morandi, 30, 20097, San Donato Milanese, Milan, Italy
e-mail: giovanni.meola@unimi.it

R. Cardani
Department of Molecular Biology and Biotechnologies, University of Milan, Milan, Italy

R. Cardani
CMN-Neuromuscular Disease Center, IRCCS Policlinico San Donato, University of Milan, Milan, Italy

Introduction

Muscle biopsy plays an important role in evaluation of patients with neuromuscular disease, and it is required to provide definitive diagnosis in many neuromuscular disorders. In addition, muscle biopsy can sometimes help to distinguish between a neurogenic or myogenic disorder, and it may give important information on the course of the disease (acute or chronic) and on disease stage and progression.

At present, muscle biopsy is necessary for diagnosis of several categories of muscle diseases, including hereditary disorders (i.e., muscular dystrophies, myotonic dystrophies) and acquired myopathies, such as inflammatory myopathies, and toxic and drug-induced myopathies (Table 1).

Muscle biopsy techniques

Muscle biopsies can be performed through open or needle technique.

Table 1 Classification of primary muscle disease

Category	Muscle disorder
Hereditary disorders	Muscular dystrophies
	Myotonic dystrophies
	Congenital myopathies
	Channelopathies
	Primary metabolic disorders
	Carbohydrate metabolism
	Lipid metabolism
Acquired myopathies	Inflammatory myopathies
	Toxic/drug-induced myopathies
	Endocrine myopathies
	Systemic illness-associated myopathies

Open muscle biopsy

Open muscle biopsy consists in an incision of 4–6 cm along the long axis of the limb through the skin, subcutaneous tissue, and muscle fascia. After the muscle sample is excised, absorbable sutures close the tensor fascia lata and the subcutaneous tissue. The technique is performed under local anesthesia with previous injection of 1–2 mL lidocaine into the skin and subcutaneous tissue and then into subcutaneous tissue and muscle fascia. It is important not to infiltrate the muscle with lidocaine to avoid alteration of biochemical results. The complications of the procedure include muscle herniation, hematoma formation, wound dehiscence, and infection.

Muscle needle biopsy

Muscle needle biopsy was developed by Bergström in 1962 [1] as an alternative to open biopsy. After local anesthesia, a 4–5-mm stab incision is made in the skin and underlying fascia with a surgical blade (#11) to facilitate passage of the biopsy needle. When it is inside the muscle, the cutting window is opened by sliding the inner cannula that it is pushed back to cut the sample. Some clinicians repeat the procedure to obtain more muscle samples while others prefer to rotate the needle to obtain different samples with one needle insertion [2]. During the procedure the patient can feel deep pressure due to the pump. Sometimes, if the muscle is not relaxed, they can complain of a cramp sensation. Derry et al. [3] assessed patient perception of the procedure and found a mild to moderate degree of discomfort. Patients with inflammatory myopathies complain of greater discomfort than others [3].

At the beginning, the major limitation of the needle biopsy technique was sample size, which was smaller than that obtained with open biopsy. Over the years, several modifications to increase the sample size have been introduced, more specifically the application of suction during the procedure [4–6]. Melendez et al. [7], using a 6-mm Bergström needle with wall suction technique on 55 subjects, obtained mean biopsy sample of 233 mg compared with 25 mg obtained with the original technique. Other limitations of needle biopsy are the impossibility of direct visualization of the sampling site and difficulty in identifying the correct orientation of the fibers of the sample obtained due to its small size. However, a recent study on more than 13,000 patients demonstrated that, using needle biopsy, it is possible to obtain an appropriate sample in >99% of cases for the analyses required [2]. Moreover, needle biopsy is a less invasive procedure than open biopsy and presents low complication rates, and the scar is shorter. Needle biopsy is particularly indicated for diagnosis of neuromuscular disease in infancy and childhood or in inflammatory myopathy to evaluate disease progression and response to treatment.

Muscle selection

The selection of the muscle to biopsy is determined by the distribution of muscle weakness. It should be a muscle affected by the disease but not be too weak or too atrophic, since in end-stage muscle where loss of myofibers is severe, muscle tissue is replaced by fibrovascular and adipose tissue [8]. More specifically, in chronic disease it should be taken from a muscle with moderate but not severe weakness, while in acute disease a principally involved muscle should be preferred.

In some cases it could be useful to perform sonographically guided percutaneous muscle biopsy or magnetic resonance imaging to assess muscle composition, especially in myositis where the distribution of inflammation inside the muscle may be heterogeneous (patchy) [9, 10].

Usually in case of proximal muscle involvement the quadriceps (rectus femoris or vastus lateralis) and the biceps are biopsied. Moreover, the quadriceps is considered relatively safe for needle biopsy because major nerves and blood vessels are far from the biopsy site. In contrast, needle biopsy of the biceps may be more dangerous, therefore some centers prefer to practice open biopsy of the biceps. Another muscle usually biopsied is the deltoid, especially under suspicion of mitochondrial disorder. Finally, to investigate distal myopathy, it may be useful to perform biopsy of the tibialis anterior muscle, which is also easy to perform by needle technique.

Specimen preparation

Diagnostic muscle biopsy consists of two types of fresh specimens: some for histopathological studies, and others

for biochemical or genetic analysis. Sometimes a sample is required to be fixed.

The samples must be immediately transported to the pathology laboratory for processing to prevent loss of enzymatic reactivity, DNA depletion or RNA degradation. Since it is an invasive investigation, it is important that the biopsy procedure and subsequent laboratory processing of the specimen are well performed to obtain an optimal sample for diagnosis. Ideally the specimens should be frozen immediately after excision, and the correct methods of freezing as well as the storage of frozen biopsies are crucial for histopathological or biomolecular diagnosis [11]. They can be sent to the laboratory on saline-moistened gauze and kept cool in a sealed container on ice. Rapid freezing at ultracold temperature and good storage of muscle specimens are essential to avoid morphologic alterations and degradation of nucleic acids. Indeed, slow tissue freezing can yield a riddled sample as a consequence of formation of ice crystal artifacts. When the specimens arrive in the laboratory, fresh samples are mounted in the appropriate orientation on cork by using a cryostat embedding medium, such as OCT compound, with fibers oriented longitudinally in the vertical plane and snap-frozen by immersing it into isopentane prechilled in liquid nitrogen. Frozen cryostat cross-sections (5–8 μm) are cut from this sample for histological, histochemical, and immunohistochemical analysis. The other fresh samples are snap-frozen in prechilled isopentane without using mounting medium which could interfere with the results of biochemical or genetic analysis.

Sometimes a fixed specimen may be useful for routine light microscopy or for electron microscopy (EM). For light microscopy, the preferred fixative is 4% paraformaldehyde; however, if it is not available, a good alternative is 10% neutral buffered formalin. For EM, a small piece of muscle can be fixed in 3% glutaraldehyde.

A battery of stainings is performed on every frozen muscle biopsy arriving in the pathology laboratory. These stainings are important in the evaluation of fiber atrophy, morphological and structural changes, and metabolic disorders [8]. The most common stainings which are useful for diagnosis of myopathies will be described here.

Hematoxylin and eosin (H&E) and the modified Gomori trichrome stain [12] are routine histological stains for evaluation of basic tissue organization and cellular structure. Moreover, Gomori trichrome is important for diagnosis of mitochondrial disorders, inclusion body myositis, and nemaline myopathy [13–15] (Fig. 1a, b).

Since muscle is composed of two main fiber types, type 1 and type 2, a myosin adenosine triphosphatase (ATPase) stain at multiple pH levels is performed to demonstrate the different fiber types [16]. Indeed, several muscular diseases affect one type or the other, causing atrophy of either type 1 or type 2 fibers (Fig. 1d). Moreover, neurogenic disorders can alter the distribution of both fiber types.

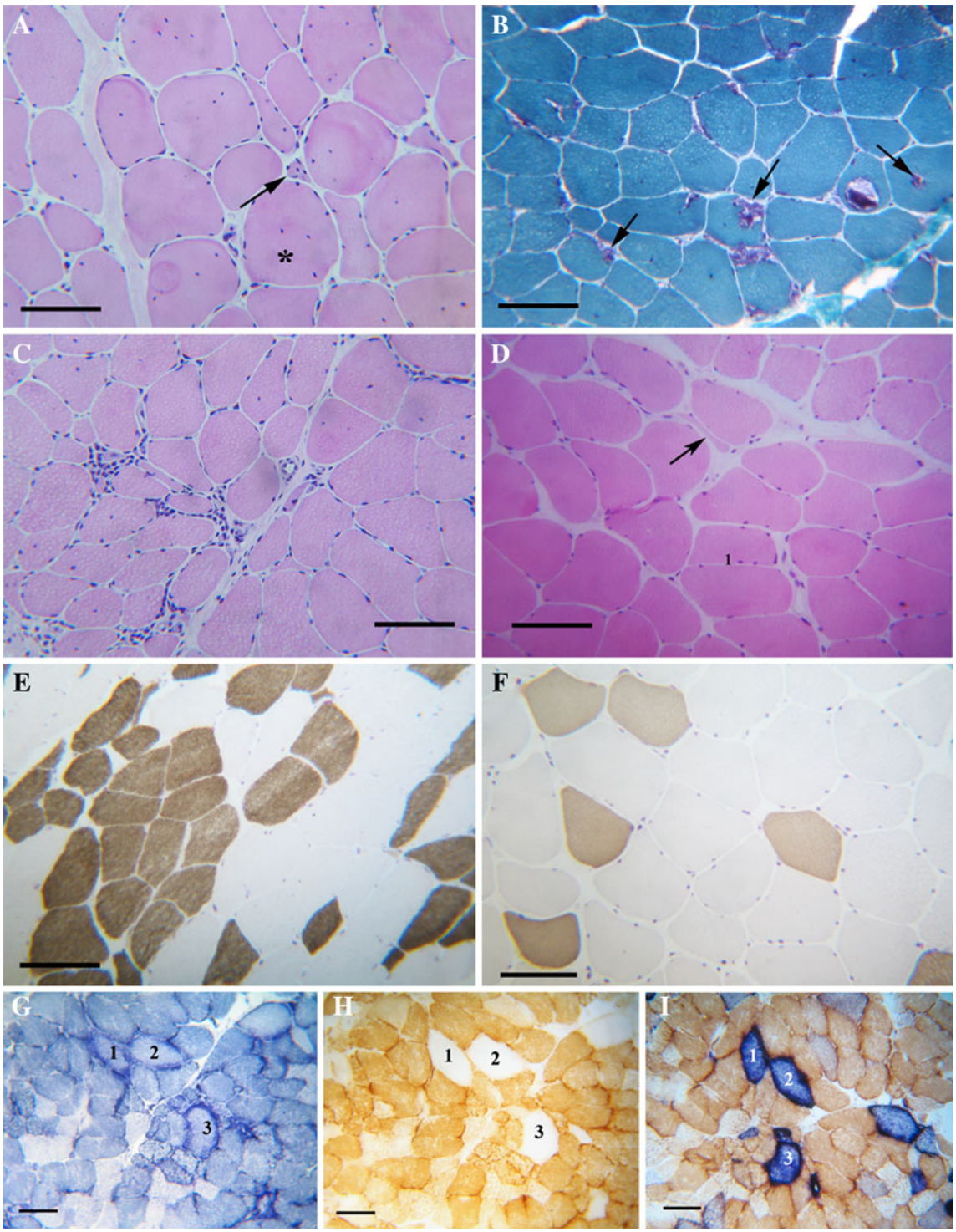
Periodic acid-Schiff (PAS) reaction is used to stain glycogen and other polysaccharides, and it is useful for diagnosis of glycogen storage diseases. Oil-red-O or Sudan Black are used for fat staining, which is normally present in muscle fibers. However, an abnormal amount or distribution of lipid is present in carnitine deficiency and in some mitochondrial disorders.

The activity of a group of enzymes which are present in mitochondria and endoplasmic reticulum is demonstrated by nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR). This staining is useful for neurogenic atrophy, mitochondrial disorders, central core disease, and for detecting alterations of myofiber intracellular structure. Moreover, the activities of two mitochondrial enzymes, succinic dehydrogenase (SDH) and cytochrome oxidase (COX), can be assessed. These histochemical stainings are often combined, and fibers that are positive for both COX and SDH appear taupe, resulting from the combination of the brown color of the stain for COX with the blue color of the SDH stain. Blue fibers are those that stain for SDH activity alone because of absence of COX activity (ragged red fibers on trichrome stain) (Fig. 1h). Lysosomal activity is evaluated by acid phosphatase staining since it is important for certain metabolic and some toxic disorders.

When the clinical history and the results of the evaluation of the routine stainings require, further stainings may be carried out on the frozen sample.

The increasing availability of antibodies against muscular proteins allows pathologists to use immunohistochemistry as a diagnostic tool for many muscle diseases (Table 2). Indeed, immunohistochemistry may be used for both identification of normal antigens in skeletal muscle and their over- or underexpression or mislocalization in corresponding myopathies. Currently, three categories of muscle diseases are characterized by using diagnostic antibodies: dystrophic, congenital/structural, and inflammatory myopathies [17].

Elevated levels of serum creatine kinase (CK; >1,000 IU/L) often indicate muscle disease with a primary problem in the muscle membrane. A number of sarcolemmal proteins have been identified whose deficiency causes different forms of limb-girdle dystrophy, including dysferlin [18], sarcoglycan [19, 20], calpain [21], and caveolin [22] (Figs. 2a and 3). For these muscular dystrophies, immunohistochemical studies using antibody against specific antigens can be performed to verify the expression of these proteins. In addiction to or alternatively to immunohistochemistry, biochemical analysis, i.e., Western blotting analysis, may lead to a specific diagnosis. Inflammatory myopathies are the largest group of acquired



muscle diseases. To identify autoimmune or inflammatory myopathies, immunohistochemistry against major histocompatibility class I, also known as human leukocyte antigens (HLA I), is often used, since it has been demonstrated that this protein is strongly upregulated in inflammatory myopathies [23–25]. In particular, since in polymyositis the inflammatory infiltrates are typically dominated by CD8+ T lymphocytes whereas in dermatomyositis CD4+ T lymphocytes are predominant, immunohistochemistry can be used to distinguish between these two inflammatory disorders (Fig. 2b). In sporadic inclusion body myositis (s-IBM), a chronic inflammatory myopathy, abnormal muscle fibers containing characteristic filamentous inclusions are present in addition to lymphocytic

◀ **Fig. 1** Histological and histochemical stainings of transverse sections of frozen muscle biopsy. **a** Myotonic dystrophy type 1: hematoxylin and eosin (H&E). High fiber size variability with several atrophic (*arrow*) and hypertrophic (*asterisk*) fibers. An increase in internal nuclei is also evident. *Bar* 80 μm. **b** Hypokalemic periodic paralysis: Gomori trichrome. Note the presence of several vacuoles (*arrows*) in myofibers. *Bar* 80 μm. **c** Inflammatory myopathy: hematoxylin and eosin (H&E). Endomysial lymphocytic inflammatory infiltrate among myofibers that show increased size variability. *Bar* 80 μm. **d** HyperCKemia: hematoxylin and eosin (H&E). In this cross-section myofiber hypertrophy, atrophy (*arrow*), and splitting (1) are present. *Bar* 80 μm. **e** Neurogenic disorder: myosin adenosine triphosphatase (ATPase) pH 4.3. Cluster of myofibers composed exclusively of type 1 fibers stained brown. *Bar* 80 μm. **f** HyperCKemia (same patient as in **d**): myosin adenosine triphosphatase (ATPase) pH 4.3. Predominance of type 2 fibers (unstained). Only a few myofibers are of type 1 (*brown*). *Bar* 80 μm. **g–i** Mitochondrial myopathy: combined staining for activities of cytochrome oxidase (COX) and succinic dehydrogenase (SDH) (**g**), COX activity stain (**h**), and nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR) stain (**i**). In **g** several *blue fibers* have a subsarcolemmal dense blue stain representing aggregates of mitochondria (1, 2, and 3). All of the fibers negative for COX activity (**h**) are blue fibers in the COX/SDH preparation (**i**). These fibers correspond to the ragged red fibers on Gomori trichrome stain. The *dark-blue* rim is also evident in COX/SDH stain (**i**). *Bar* 80 μm

inflammation. The abnormal fibers contain vacuoles well visualized by Gomori trichrome stain and amyloid foci positive with Congo red, thioflavin-s, and crystal violet. However, these amyloid deposits can often be missed since they are very small and present in only a few muscle fibers. Thus, for s-IBM diagnosis, immunocytochemical staining for the presence of phosphorylated tau in the form of paired helical filaments utilizing SMI-31 monoclonal antibody is recommended (Fig. 2c) [26, 27]. Immunostaining of type 1 and type 2 myofibers using antibodies against different myosin heavy chains is an alternative and more sensitive method than ATPase for myofiber typing [28]. Recently, myotonic dystrophy type 2/proximal myotonic myopathy (PROMM) has been defined as "a disease of type 2 fibers" since both fiber atrophy and central nucleation selectively affect type 2 fibers [29, 30] (Fig. 2d). It appears that it is of particular importance for definitive diagnosis of DM2 to distinguish between the two types of fibers.

It is noteworthy that most of the analysis described can be performed on frozen but not on fixed material. However, fixed and paraffin-embedded specimens maintain more cytological details and are more useful to detect myofiber necrosis, to determine the type of inflammatory infiltrate, and to examine the structure of vessel walls, making these specimens perfect for evaluating, for example, inflammatory myopathies or vasculitis. EM on muscle biopsy samples is not a routine procedure, but it is performed when the pathologist decides that EM might contribute significantly

Table 2 Primary and secondary protein defects in muscle disorders detectable by immunoanalysis

	Muscle disorder
Primary protein defects	
Sarcolemma	
Dystrophin	Duchenne (absent) and Becker (reduced) muscular dystrophy
Sarcoglycans	Limb-girdle muscular dystrophies 2C–D
Dysferlin	Limb-girdle muscular dystrophy type 2B
	Miyoshi muscular dystrophy
Caveolin-3	Limb-girdle muscular dystrophy type 1C
	Rippling muscle disease
	Autoimmune caveolin-3
Laminin-α2	Merosin-deficient congenital muscular dystrophy
Collagen IV	Ullrich congenital muscular dystrophy
Integrin-α7	Mild congenital dystrophy
Nuclear membrane	
Emerin	Emery–Dreifuss muscular dystrophy
Sarcoplasmic reticulum	
SERCA1	Brody disease
Cytoskeleton	
Plectin	Epidermolysis bullosa with muscular dystrophy
Enzymes	
Calpain-3	Limb-girdle muscular dystrophy type 2a
Protein accumulation	
Actin	Congenital actinopathy/nemaline myopathy
Myosin	Hyaline body myopathies
Desmin	Desminopathies
Myotilin	Myofibrillar myopathy
Secondary protein defects	
Utrophin	Duchenne/Becker muscular dystrophy
	Limb-girdle muscular dystrophies
Sarcoglycans	Limb-girdle muscular dystrophies 2C-F
	Duchenne/Becker muscular dystrophy
β-Dystroglycan	Duchenne/Becker muscular dystrophy
Neuronal nitric oxide synthase	Duchenne/Becker muscular dystrophy
	Neurogenic disorders
Laminin-α2	Congenital muscular dystrophies
Laminin-β1	Limb-girdle muscular dystrophy type 2I
	Emery–Dreifuss muscular dystrophy (autosomal dominant form)
	Bethlem myopathy
Laminin-α5	Congenital muscular dystrophies
	Neurogenic disorders
Major histocompatibility complex class I	Inflammatory myopathies
	Limb-girdle muscular dystrophy type 2B
Desmin accumulation	Myofibrillar myopathies
Phosphorylated tau accumulation	Inclusion body myositis
	Myofibrillar myopathies

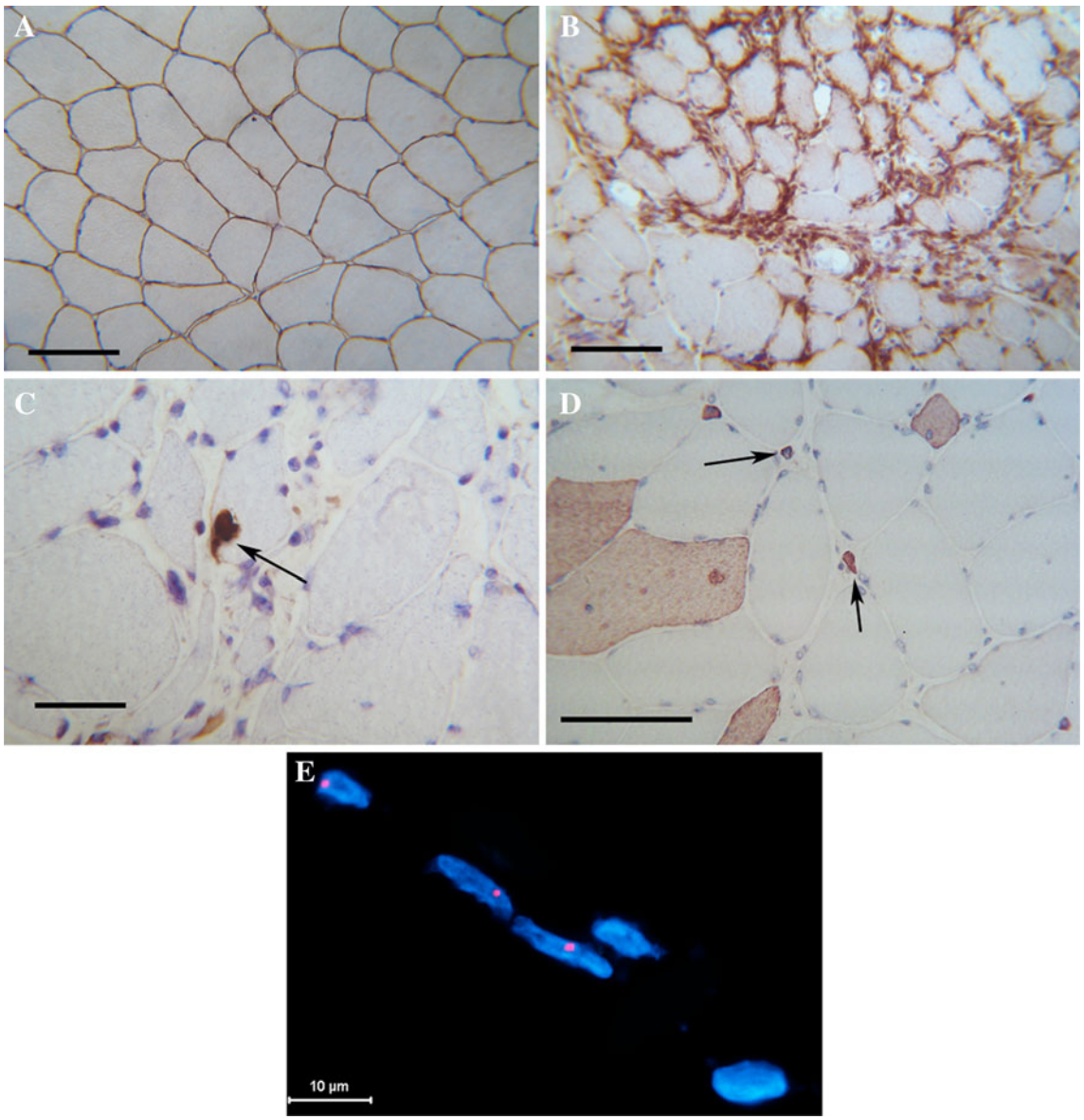


Fig. 2 Immunohistochemical and fluorescence in situ hybridization (FISH) stainings of transverse sections of frozen muscle biopsy. **a** Normal muscle: dystrophin immunohistochemistry. Sample of normal muscle stained with an antibody to C-terminal region of dystrophin shows the normal subsarcolemmal localization of this protein demonstrated by the linear peripheral brown staining of every myofiber. *Bar* 80 µm. **b** Dermatomyositis: immunohistochemistry for CD4 (T lymphocytes) with hematoxylin counterstain. T lymphocytes (*brown cells*) infiltrate the endomysial connective tissue. *Bar* 80 µm. **c** Inclusion body myositis: immunohistochemistry for SMI-31. Characteristic inclusion stained with SMI-31 antibody (*arrow*) in an abnormal myofiber. *Bar* 80 µm. **d** Myotonic dystrophy type 2: fast myosin immunohistochemistry. The immunostaining (*brown*) demonstrates the presence of very atrophic fibers which are fast positive, i.e., type 2 fibers (*arrows*). *Bar* 100 µm. **e** Myotonic dystrophy type 2: FISH. Direct visualization of mutant messenger RNA (mRNA) containing CCUG repeat (*red spots*) in myonuclei [4',6-diamidino-2-phenylindole (DAPI), *blue*]

to determining a specific diagnosis on the basis of clinical history and the results of light microscopy evaluations [31].

The development of molecular biology and its application to muscle diseases has led to the identification of gene defects in many inherited neuromuscular diseases and therefore to accurate and specific diagnosis (Table 3). The best example of this is Duchenne muscular dystrophy (DMD) and the discovery in the late 1980s of the gene at locus Xp21 whose mutation causes deficiency of dystrophin, an essential protein in muscle fibers (Fig. 3) [32]. Some investigators give first preference to noninvasive molecular analysis versus microscopic study of invasive muscle biopsy [33], for example in cases where clinical and genetic history is highly suggestive for DMD but confirmation is necessary for differential diagnosis from Becker or other forms of muscular dystrophy. Others

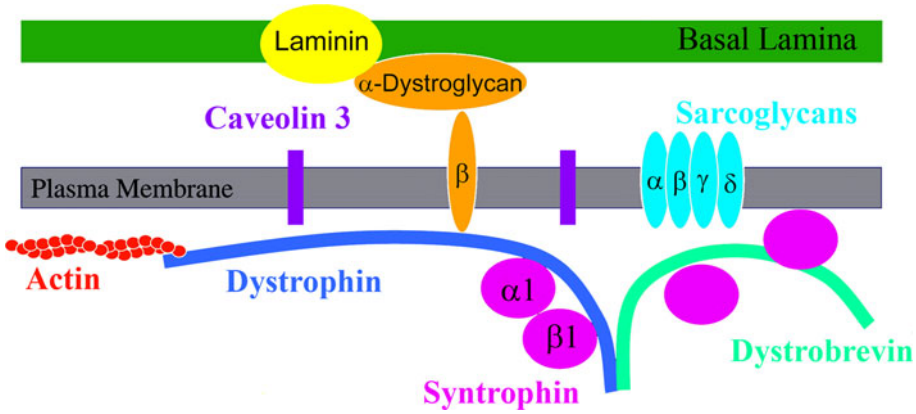


Fig. 3 Simplified scheme of the extracellular matrix and sarcolemmal proteins

Table 3 Classification of some muscle diseases according to the molecular defect

Myopathies	Muscular disorder (OMIM)	Protein (Gene MIM) Gene locus	Definitive diagnostic method
Dystrophinopathies	Duchenne muscular dystrophy (DMD #310200)	Dystrophin (300377) Xp21.2	Immunohistochemistry; western blot; molecular testing
	Becker muscular dystrophy (BMD #300376)	Dystrophin (300377) Xp21.2	Immunohistochemistry; western blot; molecular testing
	Cardiomyopathy dilated 3B (CMD3B #302045)	Dystrophin (300377) Xp21.2	Immunohistochemistry; western blot; molecular testing
Sarcoglycanopathies	Limb-girdle type 2D (LGMD2D; #608099	Alpha-sarcoglycan (SGCA; 600119) 17q12–q21.33	Immunohistochemistry; western blot; molecular testing
	Limb-girdle type 2E (LGMD2E; #604286)	Beta-sarcoglycan (SGCB; 600900) 4q12	Immunohistochemistry; western blot; molecular testing
	Limb-girdle type 2F (LGMD2F; #601287)	Sarcoglycan-delta (SGCD; 601411) 5q33	Immunohistochemistry; western blot; molecular testing
	Limb-girdle type 2C (LGMD2C; #253700)	Gamma-sarcoglycan (SGCG; 608896) 13q12	Immunohistochemistry; western blot; molecular testing
Dysferlinopathies	Limb-girdle type 2B (LGMD2B; #253601)	Dysferlin (DYSF; 603009) 2p13.3–p13.1	Immunohistochemistry; western blot; molecular testing
	Miyoshi muscular dystrophy (MMD1#254130)	Dysferlin (DYSF; 603009) 2p13.3–p13.1	Immunohistochemistry; western blot; molecular testing
Caveolinopathies	Limb-girdle type 1C (LGMD1C; #607801)	Caveolin-3 (CAV3; 601253) 3p25	Immunohistochemistry; western blot; molecular testing
	Rippling muscle disease (RMD; #606072)	Caveolin-3 (CAV3; 601253) 3p25, 1q41	Immunohistochemistry; western blot; molecular testing
Ion channelopathies	Myotonia congenita dominant, Thomsen disease (DMC #160800)	Chloride channel-1, skeletal muscle (CLCN1; 118425) 7q35	Molecular testing
	Myotonia congenita recessive, Becker disease (RMC #160800)	Chloride channel-1, skeletal muscle (CLCN1; 118425) 7q35	Molecular testing

Table 3 continued

Myopathies	Muscular disorder (OMIM)	Protein (Gene MIM) Gene locus	Definitive diagnostic method
	Potassium-aggravated myotonia (PAM #608390)	Sodium channel, voltage-gated (SCN4A; 603967) 17q23.1–q25.3	Molecular testing
	Paramyotonia congenita (PC #168300)	Sodium channel, voltage-gated (SCN4A; 603967) 17q23.1–q25.3	Molecular testing
	Hyperkalemic periodic paralysis (HYPP #170500)	Sodium channel, voltage-gated (SCN4A; 603967) 17q23.1–q25.3	Molecular testing
	Hypokalemic periodic paralysis type 1 (HOKPP #613345)	Calcium channel, voltage-dependent (CACNA1S; 114208) 1q32	Molecular testing
	Hypokalemic periodic paralysis type 2 (HOKPP2 #613345)	Sodium channel, voltage-gated (SCN4A; 603967) 17q23.1–q25.3	Molecular testing
Repeat expansion myopathies	Myotonic dystrophy type 1 (DM1#160900)	Dystrophia myotonica protein kinase (DMPK; 605377) 19q13.2–q13.3	Molecular testing
	Myotonic dystrophy type 2 (DM2#160900)	Zinc finger 9 gene (ZNF9; 116955) 3q13.3–q24	Molecular testing; in situ hybridization
Emerin deficiency	Emery–Dreifuss muscular dystrophy (EDMD1 #310300)	Emerin (EMD; 300384) Xq28	Molecular testing
Laminin A deficiency	Emery–Dreifuss muscular dystrophy (EDMD4 #612998)	Lamin A/C (LMNA; 150330) 1q21.2	Molecular testing
Other muscular dystrophies	Facioscapulohumeral muscular dystrophy (FSHD #158900)	Facioscapulohumeral muscular dystrophy-1A (FSHMD1A; 158900) 4q35	Molecular testing
	Oculopharyngeal muscular dystrophy (OPMD #164300)	Poly(A)-binding protein, nuclear 1 (PABPN1; 602279) 14q11.2–q13	Molecular testing
	Congenital muscular dystrophy (MDC1A #607855)	Laminin alpha-2 gene (LAMA2; 156225) 6q22–q23	Immunohistochemistry; western blot; molecular testing
	Tibial muscular dystrophy (TMD #600334)	Titin (TTN; 188840) 2q31	Molecular testing

investigators choose demonstration of dystrophin deficiency by immunohistochemistry or Western blot analysis on muscle biopsy. In selected cases it is possible to demonstrate the genetic alteration directly on muscle section using in situ hybridization (ISH). An example of this is represented by myotonic dystrophy type 2, a muscular dystrophy caused by a CCTG repeat expansion in intron 1 of the zinc finger protein 9 gene on chromosome 3 [34, 35]. Due to the extremely large size of the CCTG expansion and the extensive somatic instability [36, 37], standard molecular analysis such as Southern analysis and polymerase chain reaction (PCR) fail to detect the DM2 expansion. To date, a three-step method based on repeat primed (RP) PCR with 99% accuracy is recommended to determine the presence of DM2 mutation [38]. Several alternative highly specific and sensitive methods have been developed in addition to RP-PCR; however, they are expensive, not available as routine diagnostic test, and very time-consuming. For these reasons, muscle biopsy represents an essential tool for definitive biomolecular diagnosis of DM2 which may be obtained in few hours by ISH using a specific probe which allows direct visualization of mutant

mRNA containing CCUG repeat on muscle sections [39, 40] (Fig. 2e). However, there are myopathies that do not need muscle biopsy for definitive diagnosis, such as some congenital and limb-girdle dystrophies, myotonic dystrophy type 1, certain mitochondrial disorders, and periodic paralyses. In these cases, genetic tests are performed on a blood sample. Myotonic dystrophy type 1 is definitively diagnosed by means of genetic testing on a sample of blood, which reveals a characteristic increase in the number of CTG triplet repeats in the *DMPK* gene on chromosome 19 [41–43].

It should be noted that, from a skeletal muscle biopsy, it is possible to isolate the progenitors of muscle fibers, the satellite cells. Skeletal muscle satellite cells are quiescent mononucleated myogenic cells, located between the sarcolemma and basement membrane of terminally differentiated muscle fibers [44]. These are normally quiescent in adult muscle, but act as a reserve population of cells, able to proliferate in response to injury and give rise to regenerated muscle and to more satellite cells [45]. Isolated satellite cells are very useful for in vitro studies. Indeed, muscle cell cultures provide a system for studying the growth and differentiation of muscle cells in a controlled environment.

Conclusions

In addition to the well-defined pathological conditions that occur in various neuromuscular disorders, skeletal muscle may also be directly or indirectly involved in many acute or chronic diseases. Muscle biopsy may reveal striking and unexpected changes in situations where involvement of the muscle is not clinically apparent.

The molecular era has led to the identification of many gene defects and proteins responsible for neuromuscular disorders. Several proteins of muscle fibers, the extracellular matrix, the plasma membrane, the cytoskeleton, the Golgi apparatus, the internal membrane systems, nuclei, myofibrils, neuromuscular junction, and the cytosol have been found. The wide spectrum of defective proteins that have been identified and the interactions between them have challenged the traditional classification of neuromuscular disorders based on clinical features. Neuromuscular diseases are now referred to on the basis of the protein defect, such as dystrophinopathy, sarcoglycanopathy, and actinopathy, and according to the pathogenic mechanisms.

When the pathologist is asked to evaluate a muscle biopsy, much of the interpretation is based on his previous experience and the recognition of similarities between the biopsy and muscle from known diseases. In such instances, such as an advances dystrophy or spinal muscular atrophy, the changes may be striking and unequivocal. In others, the changes may be more subtle and a systematic approach is required in the evaluation and interpretation. Once the pathology is defined, correlation of the clinical features is essential and the pathology must be interpreted in light of this. Muscle biopsy is a useful technique which can make an enormous contribution in the field of neuromuscular disorders but that should be considered and interpreted together with family history, clinical history and presentation, and the result of any other investigation. There are considerable advantages if the clinician assessing the patients can also review the biopsy with the pathologist and then provide a comprehensive diagnosis of the patient. This is our policy and gives continuity between the clinical diagnosis and other investigations.

Conflict of interest None.

References

1. Bergström J (1962) Muscle electrolytes in man. Scand J Clin Lab Med 14:511–514
2. Tarnopolsky MA, Pearce E, Smith K, Lach B (2011) Suction-modified Bergström muscle biopsy technique: experience with 13, 500 procedures. Muscle Nerve 43:717–725
3. Derry KL, Nicolle MN, Keith-Rokosh JA, Hammond RR (2009) Percutaneous muscle biopsies: review of 900 consecutive cases at London Health Sciences Centre. Can J Neurol Sci 36:201–206
4. Evans WJ, Phinney SD, Young VR (1982) Suction applied to a muscle biopsy maximizes sample size. Med Sci Sports Exerc 14:101–102
5. Hennessey JV, Chromiak JA, DellaVentura S, Guertin J, MacLean DB (1997) Increase in percutaneous muscle biopsy yield with a suction-enhancement technique. J Appl Physiol 82:1739–1742
6. Greig PD, Askanazi J, Kinney JM (1985) Needle biopsy of skeletal muscle using suction. Surg Gynecol Obstet 160:466–498
7. Melendez MM, Vosswinkel JA, Shapiro MJ, Gelato MC, Mynarcik D, Gavi S, Xu X, McNurlan M (2007) Wall suction applied to needle muscle biopsy—a novel technique for increasing sample size. J Surg Res 142:301–303
8. Dubowitz V, Sewry C (2007) Muscle Biopsy: A Practical Approach, 3rd edn. Saunders/Elsevier, Philadelphia, PA
9. O'Sullivan PJ, Gorman GM, Hardiman OM, Farrell MJ, Logan PM (2006) Sonographically guided percutaneous muscle biopsy in diagnosis of neuromuscular disease: a useful alternative to open surgical biopsy. J Ultrasound Med 25:1–6
10. Connor A, Stebbings S, Anne HN, Hammond-Tooke G, Meikle G, Highton J (2007) STIR MRI to direct muscle biopsy in suspected idiopathic inflammatory myopathy. J Clin Rheumatol 13:341–345
11. Meola G (2005) Advanced microscopic and histochemical techniques: diagnostic tools in the molecular era of myology. Eur J Histochem 49:93–96
12. Engel WK, Cunningham GG (1963) Rapid examination of muscle tissue: an improved trichrome method for fresh frozen biopsy sections. Neurology 13:919–923
13. Taylor RW, Schaefer AM, Barron MJ, McFarland R, Turnbull DM (2004) The diagnosis of mitochondrial muscle disease. Neuromuscul Disord 14:237–245

14. Needham M, Mastaglia FL (2007) Inclusion body myositis: current pathogenetic concepts and diagnostic and therapeutic approaches. Lancet Neurol 6:620–631
15. Sewry CA (2008) Pathological defects in congenital myopathies. J Muscle Res Cell Motil 29:231–238
16. Round JM, Matthews Y, Jones DA (1980) A quick, simple and reliable histochemical method for ATPase in human muscle preparations. Histochem J 12:707–710
17. Vogel H, Zamecnik J (2005) Diagnostic immunohistology of muscle diseases. J Neuropathol Exp Neurol 64:181–193
18. Bashir R, Britton S, Strachan T, Keers S, Vafiadaki E, Lako M, Richard I, Marchand S, Bourg N, Argov Z, Sadeh M, Mahjneh I, Marconi G, Passos-Bueno MR, Moreira Ede S, Zatz M, Beckmann JS, Bushby K (1998) A gene related to Caenorhabditis elegans spermatogenesis factor fer-1 is mutated in limb-girdle muscular dystrophy type 2B. Nat Genet 20:37–42
19. Duggan DJ, Manchester D, Stears KP, Mathews DJ, Hart C, Hoffman EP (1997) Mutations in the delta-sarcoglycan gene are a rare cause of autosomal recessive limb-girdle muscular dystrophy (LGMD2). Neurogenetics 1:49–58
20. Duggan DJ, Gorospe JR, Fanin M, Hoffman EP, Angelini C (1997) Mutations in the sarcoglycan genes in patients with myopathy. N Engl J Med 336:618–624
21. Richard I, Broux O, Allamand V, Fougerousse F, Chiannilkulchai N, Bourg N, Brenguier L, Devaud C, Pasturaud P, Roudaut C et al (1995) Mutations in the proteolytic enzyme calpain 3 cause limb-girdle muscular dystrophy type 2A. Cell 81:27–40
22. Minetti C, Sotgia F, Bruno C, Scartezzini P, Broda P, Bado M, Masetti E, Mazzocco M, Egeo A, Donati MA, Volonte D, Galbiati F, Cordone G, Bricarelli FD, Lisanti MP, Zara F (1998) Mutations in the caveolin-3 gene cause autosomal dominant limb-girdle muscular dystrophy. Nat Genet 18:365–368
23. Karpati G, Pouliot Y, Carpenter S (1988) Expression of immunoreactive major histocompatibility complex products in human skeletal muscles. Ann Neurol 23:64–72
24. Karpati G, Carpenter S (1993) Pathology of the inflammatory myoathies. In: Fl M (ed) Bailliere's clinical neurology. Baillere Tindall, London, pp 527–556
25. Emslie-Smith AM, Arahata K, Engel AG (1989) Major histocompatibility complex class I antigen expression, immunolocalization of interferon subtypes, and T cell-mediated cytotoxicity in myopathies. Hum Pathol 20:224–231
26. Askanas V, Engel WK (2001) Inclusion-body myositis: newest concepts of pathogenesis and relation to aging and Alzheimer disease. J Neuropathol Exp Neurol 60:1–14
27. van der Meulen MF, Hoogendijk JE, Moons KG, Veldman H, Badrising UA, Wokke JH (2001) Rimmed vacuoles and the added value of SMI-31 staining in diagnosing sporadic inclusion body myositis. Neuromuscul Disord 11:447–451
28. Raheem O, Huovinen S, Suominen T, Haapasalo H, Udd B (2010) Novel myosin heavy chain immunohistochemical double staining developed for the routine diagnostic separation of I, IIA and IIX fibers. Acta Neuropathol 119:495–500
29. Bassez G, Chapoy E, Bastuji-Garin S, Radvanyi-Hoffman H, Authier FJ, Pellissier JF, Eymard B, Gherardi RK (2008) Type 2 myotonic dystrophy can be predicted by the combination of type 2 muscle fiber central nucleation and scattered atrophy. J Neuropathol Exp Neurol 67:319–325
30. Vihola A, Bassez G, Meola G, Zhang S, Haapasalo H, Paetau A, Mancinelli E, Rouche A, Hogrel JY, Laforêt P, Maisonobe T, Pellissier JF, Krahe R, Eymard B, Udd B (2003) Histopathological differences of myotonic dystrophy type 1 (DM1) and PROMM/DM2. Neurology 60:1854–1857
31. Fernandez C, Figarella-Branger D, Meyronet D, Cassote E, Tong S, Pellissier JF (2005) Electron microscopy in neuromuscular disorders. Ultrastruct Pathol 29:437–450
32. Hoffman EP, Brown RH Jr, Kunkel LM (1987) Dystrophin: the protein product of the Duchenne muscular dystrophy locus. Cell 51:919–928
33. Muntoni F (2001) Is a muscle biopsy in Duchenne dystrophy really necessary? Neurology 57:574–575
34. Ranum LPW, Rasmussen P, Benzow K, Koob M, Day JW (1998) Genetic mapping of a second myotonic dystrophy locus. Nat Genet 19:196–198
35. Liquori CL, Ricker K, Moseley ML, Jacobsen JF, Kress W, Naylor SL, Day JW, Ranum LP (2001) Myotonic dystrophy type 2 caused by a CCTG expansion in intron 1 of ZNF9. Science 293:816–817
36. Bachinski LL, Udd B, Meola G, Sansone V, Bassez G, Eymard B, Thornton CA, Moxley RT, Harper PS, Rogers MT, Jurkat-Rott K, Lehmann-Horn F, Wieser T, Gamez J, Navarro C, Bottani A, Kohler A, Shriver MD, Sallinen R, Wessman M, Zhang S, Wright FA, Krahe R (2003) Confirmation of the type 2 myotonic dystrophy (CCTG)n expansion mutation in patients with proximal myotonic myopathy/proximal myotonic dystrophy of different European origins: a single shared haplotype indicates an ancestral founder effect. Am J Hum Genet 73:835–848
37. Day JW, Ricker K, Jacobsen JF, Rasmussen LJ, Dick KA, Kress W, Schneider C, Koch MC, Beilman GJ, Harrison AR, Dalton JC, Ranum LP (2003) Myotonic dystrophy type 2: molecular, diagnostic and clinical spectrum. Neurology 60:657–664
38. Udd B, Meola G, Krahe R, Thornton C, Ranum L, Day J, Bassez G, Ricker K (2003) Report of the 115th ENMC workshop: DM2/PROMM and other myotonic dystrophies. 3rd Workshop, 14–16 February 2003, Naarden, The Netherlands. Neuromuscul Disord 13:589–596
39. Cardani R, Mancinelli E, Sansone V, Rotondo G, Meola G (2004) Biomolecular identification of (CCTG)n mutation in myotonic dystrophy type 2 (DM2) by FISH on muscle biopsy. Eur J Histochem 48:437–442
40. Sallinen R, Vihola A, Bachinski LL, Huoponen K, Haapasalo H, Hackman P, Zhang S, Sirito M, Kalimo H, Meola G, Horelli-Kuitunen N, Wessman M, Krahe R, Udd B (2004) New methods for molecular diagnosis and demonstration of the (CCTG)n mutation in myotonic dystrophy type 2 (DM2). Neuromuscul Disord 14:274–283
41. Brook JD, McCurrach ME, Harley HG, Buckler AJ, Church D, Aburatani H, Hunter K, Stanton VP, Thirion JP, Hudson T et al (1992) Molecular basis of myotonic dystrophy: expansion of a trinucleotide (CTG) repeat at the 3′ end of a transcript encoding a protein kinase family member. Cell 68:799–808
42. Fu YH, Pizzuti A, Fenwick RG Jr, King J, Rajnarayan S, Dunne PW, Dubel J, Nasser GA, Ashizawa T, de Jong P et al (1992) An unstable triplet repeat in a gene related to myotonic muscular dystrophy. Science 255:1256–1258
43. Mahadevan M, Tsilfidis C, Sabourin L, Shutler G, Amemiya C, Jansen G, Neville C, Narang M, Barceló J, O'Hoy K et al (1992) Myotonic dystrophy mutation: an unstable CTG repeat in the 3' untranslated region of the gene. Science 255:1253–1255
44. Mauro A (1961) Satellite cell of skeletal muscle fibers. J Biophys Biochem Cytol 9:493–495
45. Seale P, Polesskaya A, Rudnicki MA (2003) Adult stem cell specification by Wnt signaling in muscle regeneration. Cell Cycle 2:418–419