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Myopathology in times of modern imaging

¹Department of Paediatric Neurology, Neuromuscular Service, Evelina's Children Hospital, Guy's & St. Thomas' Hospital NHS Foundation Trust, London, UK; ²Randall Division of Cell and Molecular Biophysics, Muscle Signalling Section, and; ³Department of Clinical and Basic Neuroscience, IoPPN, King's College, London, UK;

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Address for correspondence:

Dr Heinz Jungbluth
Children's Neurosciences Centre,
F01 – Staircase D South Wing,
St Thomas' Hospital,
Westminster Bridge Road,
London SE1 7EH,
United Kingdom
0044 20 71883998
e-mail: Heinz.Jungbluth@gstt.nhs.uk

ABSTRACT

Over the last two decades muscle (magnetic resonance) imaging has become an important complementary tool in the diagnosis and differential diagnosis of inherited neuromuscular disorders, particularly in conditions where the pattern of selective muscle involvement is often more predictive of the underlying genetic background than associated clinical and histopathological features.

Following an overview of different imaging modalities, the present review will give a concise introduction to systematic image analysis and interpretation in genetic neuromuscular disorders. The pattern of selective muscle involvement will be presented in detail in conditions such as the congenital or myofibrillar myopathies where muscle imaging is particularly useful to inform the (differential) diagnosis, and in disorders such as Duchenne or fascioscapulohumeral muscular dystrophy where the diagnosis is usually made on clinical grounds but where detailed knowledge of disease progression on the muscle imaging level may inform better understanding of the natural history. Utilizing the group of the congenital myopathies as an example, selected case studies will illustrate how muscle MRI can be used to inform the diagnostic process in the clinico-pathological context. Future developments, in particular concerning the increasing use of whole body MRI protocols and novel quantitative fat assessments techniques potentially relevant as an outcome measure, will be briefly outlined.

INTRODUCTION

The diagnostic approach to inherited neuromuscular disorders is a complex process with the ultimate aim of establishing an unequivocal genetic explanation for the patient presenting with muscle weakness to the neuromuscular clinic. A comprehensive personal and family history, detailed clinical assessment, CPK level determination and neurophysiological studies have traditionally provided the cornerstones of this process, and the context for the interpretation of histopathological features on muscle biopsy.

Over the last two decades, muscle imaging has become an important modality in neuromuscular diagnostics, based on the observation that different genetic defects do not affect all muscles equally and that distinct patterns of selective muscle involvement may thus point to a specific genetic background. Muscle imaging has become particularly relevant in neuromuscular disorders such as the congenital myopathies or the limb girdle muscular dystrophies where the same histopathological feature can be caused by a wide range of different mutations, or in heterogeneous syndromes such as the rigid spine syndrome (RSS) where identical clinical features do not offer any immediate clues as to the underlying genetic defect. Muscle imaging is less important as a diagnostic tool in conditions such as Duchenne muscular dystrophy, myotonic dystrophy or FSHD where the diagnosis can usually be readily made on clinical grounds, but is increasingly applied also in these conditions to monitor fatty muscle replacement more objectively through utilization of new MRI techniques. Although due to recent rapid advances genetic testing is performed increasingly early in the diagnostic process, muscle imaging is thus likely to retain its current role also in future, if only to ascertain the relevance of different genetic variants identified on massive parallel sequencing, or to monitor disease progression.

The following review will summarize the role of muscle imaging in the approach to neuromuscular disorders, focussing on inherited and myopathic conditions. The review will not cover acquired (in particular inflammatory) and neurogenic conditions, areas where muscle imaging does play a definite role but for which the reader is referred to other topical specialist reviews [1, 2]. Different imaging modalities will be summarized, a structured approach to muscle imaging analysis will be outlined and typical patterns of selective muscle involvement in common inherited neuromuscular disorders will be presented. Concise case studies utilizing the congenital myopathies as an example will illustrate how muscle imaging can be put into clinical practice, to aid interpretation of histopathological features or even substitute for a muscle biopsy where this cannot be performed. Potential and challenges for the future will be summarized in a concluding paragraph.

IMAGING MODALITIES IN INHERITED MYOPATHIES

Selective muscle involvement in inherited myopathies was originally demonstrated on early ultrasound, CT and MRI studies in the 1980s and 1990s. Although there are obvious associations between histopathological features on muscle biopsy and findings with different imaging modalities (Figure 1), there is clearly a lack of more comprehensive comparative studies, for example systematically correlating findings on muscle ultrasound and muscle MR imaging. Considering that atrophy and replacement of muscle tissue with fat and connective tissue are also features of the normal aging process, distinguishing the latter from genuinely myopathic changes may pose a considerable challenge, particularly in older patients, whatever imaging modality applied.

Muscle ultrasound (US) was one of the first imaging modalities applied in the investigation of neuromuscular disorders. On muscle US imaging (for review, [3-5]), normal muscle tissue

Muscle imaging and myopathology

has low echogenicity and appears therefore dark, whereas in myopathies (and also in aging muscle and/or obese individuals), echogenicity is high and the appearance of the muscle brightens, due to fatty and/or connective tissue increases often associated with reduced echogenicity of underlying bone structures. Muscle contraction has the contrary effect and makes muscle appear darker, an observation that has to be taken into account with image interpretation. Even normal muscle never appears completely black on muscle US, due to the presence of physiological internal connective tissue structures such as the fascia that give rise to a speckled appearance on transverse sections and can be clearly visualized on longitudinal sections. Although the echogenicity increases in myopathic conditions appear more diffuse compared to the often “moth-eaten” appearance of primary neurogenic disorders, distinction can be challenging in individual patients. Main advantages of muscle ultrasound are its ready bedside availability (particularly in children) and its unique potential to capture real life pathognomic events such as fasciculation and fibrillations, but its use is limited by its inferior visualization of deeper muscle tissues compared to other imaging techniques, in particular muscle MRI.

Muscle computer tomography (CT) (for review, [4, 5]) was widely applied in early imaging studies of neuromuscular disorders but has now been largely replaced by other imaging modalities except in cases where muscle MRI may be contraindicated (for example, a patient wearing a pacemaker), mainly reflecting the associated substantial radiation exposure and relatively low sensitivity. Muscle tissue replaced by fat appears more hypodense than normal muscle tissue on CT, but the technique is inferior compared to muscle MRI in detecting more subtle myopathic, inflammatory and/or edematous changes.

The potential of muscle magnetic resonance imaging (MRI) to image healthy and diseased skeletal muscle with an unprecedented level of contrast and clarity has been recognized since the late 1980s [6]. Whilst initially mainly used for the diagnosis and monitoring of

inflammatory myopathies [1], muscle MRI (for review, [4, 5]) is now the method of choice for (diagnostic) muscle imaging of genetic neuromuscular disorders. Axial T1-weighted (turbo/fast) and T2-weighted (turbo/fast) spin echo sequences with or without fat suppression (for example, STIR) are the most frequently used imaging sequences and mirror specific histopathological features with different signal intensity changes (summarized in Table 1). Fatty and connective tissue replacement is reflected in increased signal intensity on T1-weighted images, whereas in particular (fat suppressed) T2-weighted images can help to visualize edematous and inflammatory changes that may precede more definite changes in primary inflammatory myopathies or some of the muscular dystrophies. Muscle MRI lacks radiation and offers higher resolution compared to muscle CT, and allows for better visualization of deeper muscle layers compared to muscle US. Disadvantages are the relatively longer exposures required, in particular if whole body MRI is performed, resulting in the need for sedation or general anesthesia if young children are imaged. Whilst most of the early muscle MRI studies have focussed on the lower limbs, whole body MRIs increasingly performed more recently may provide additional useful information (including information on other organs such as the heart) whose value has to be balanced with the possible sedation requirement.

Whilst most muscle MRI studies until recently had a strong diagnostic emphasis focussing on genotype-phenotype correlations on the radiological level, *novel MRI techniques* to quantify alterations of muscle fat more precisely and reproducibly are being put into practice with the ultimate aim to establish muscle MRI as a biomarker for natural history studies and therapy monitoring (for review, [7]). The most promising of these techniques is the 3-point Dixon technique [8, 9] suitable for quantification of the intramuscular fat content in an objective manner, thus allowing comparisons between different patients and the same patient at different time points in longitudinal studies. Other novel MRI techniques currently being

assessed for their practical value are magnetic resonance spectroscopy (MRS), suitable to measure water and fat concentrations (^1H), energy metabolites (^{31}P , glycogen ^{13}C) that may be relevant for certain metabolic disorders [10] but also some of the muscular dystrophies [11-13], and intracellular sodium content (^{23}Na) that may hold promise for the investigation of sodium channelopathies [14].

IMAGING INTERPRETATION IN INHERITED MYOPATHIES

As with any other diagnostic modality, only rarely can a diagnosis be established based on muscle MRI or other imaging findings alone. Considering that the number of possible patterns of selective muscle involvement is finite and some muscle groups are more frequently affected than others throughout different myopathic conditions, additional information such as histopathological evidence of a dystrophic process or an elevated CK is invaluable in assigning a certain MRI pattern to a specific genetic background.

Muscle MRI has to be based on sound knowledge of individual muscles and muscle groups, and there are several excellent publications summarizing muscle anatomy with a particular view to muscle MRI or CT interpretation (for example, [4]). Figure 2 illustrates muscle groups commonly analysed in the lower limb on the mid-thigh and the mid-calf level.

The main general questions to be considered in the interpretation of muscle MR and CT images are i) if the muscle bulk is normal or reduced, ii) if there is any increase in signal intensity suggestive of increases in fat and connective tissue and/or abnormal water content, and, if yes, iii) if there is a pattern of selective involvement suggestive of a specific genetic background. Although the question of a non-physiological increase in signal intensity is usually easily answered in the Paediatric age group, the distinction may be less straightforward in older and obese patients where similar changes may occur without being

reflective of a primary myopathic process. Other considerations in the interpretation of muscle MRI are an assessment of the amount of subcutaneous fat (in particular looking for associated lipodystrophy), and of other organs such as the heart (in particular if a muscle condition with an associated cardiomyopathy is suspected) if whole body MRI is performed. Different visual rating scales have been designed to semi-quantify the amount of abnormally increased signal within a given muscle group based on how much of muscle tissue is being replaced by abnormal signal [15-17] and are summarized in [5].

The more specific questions to be considered in the interpretation of muscle MR and CT imaging are iv) if the pattern of involvement is proximal or distal, and v) if the anterior and posterior compartments within the thighs and the legs are affected equally, or if there is anterior or posterior predominance. Considering that some muscles are more commonly spared (or affected) across different neuromuscular disorders (Table 2), involvement of a muscle that is usually spared (for example, of the rectus femoris in FSHD), or sparing of a muscle that is usually affected (for example, the quadriceps in DES-related MFM) might provide essential diagnostic clues. In addition, an assessment of the combined pattern of selective involvement at different levels (pelvis, thigh, calves) may strongly point at a specific genetic diagnosis, and is likely to be further informed through the more widespread use of whole body MR imaging. Lastly, additional unusual features (such as the “rimming” observed in *COL6*-related myopathies, or the marked lipodystrophy in laminopathies) may provide additional diagnostic clues.

A systematic approach to the differential diagnosis of muscle imaging findings and very helpful diagnostic algorithms are summarized in [4, 5].

MUSCLE IMAGING IN SPECIFIC INHERITED NEUROMUSCULAR DISORDERS

Muscle imaging has been applied through a wide range of inherited neuromuscular disorders but to variable extent and with different remit. In general, the diagnostic yield of muscle imaging is highest in stable or slowly progressive conditions with mild to moderate weakness in which selective patterns of muscle involvement can be discerned over prolonged periods of time (for example, some of the congenital myopathies), in contrast to very mild and/or episodic disorders without fixed weakness where muscle imaging may often be normal (for example, some metabolic myopathies or the periodic paralyses), or in profoundly severe disorders where muscle involvement may be so extensive that no selective involvement can be detected anymore. Muscle imaging is most useful in conditions with overlapping clinical and histopathological findings, where the clinico-pathological features do not immediately suggest a genetic diagnosis, and identification of a distinct pattern of selective muscle involvement may aid the choice of genetic testing (or variant interpretation where parallel sequencing of a large number of genes has already been performed). Muscle imaging is less important as a diagnostic tool in conditions such as Duchenne (DMD) and Becker muscular dystrophy (BMD), Fascio-Scapulo-Humeral Dystrophy (FSHD) or myotonic dystrophy (MD) that can be readily diagnosed on clinical and/or histopathological grounds, but may be useful also in these disorders if presentations are atypical, and, through application of novel MRI techniques, as a tool to objectively quantify intramuscular fat as an outcome measure. The following paragraph will outline characteristic imaging findings in selected inherited neuromuscular disorders (illustrated in Figure 3 and summarized in Table 2) (for a more detailed review, [4]).

Congenital myopathies

The congenital myopathies – Central Core Disease (CCD), Multi-Minicore Disease (MmD), Centronuclear Myopathy (CNM), Nemaline Myopathy (NM) and Congenital Fibre Type Disproportion (CFTD) – are a genetically heterogeneous group of early-onset myopathies characterized by variable degrees of weakness, a normal or only mildly elevated CK, and defined by distinct features on muscle biopsy (for review, [18]). Considering that the same genetic background may cause highly variable histopathological presentations and that, vice versa, mutations in different genes may cause the same congenital myopathy, muscle MRI is often a better predictor of the causative gene than muscle biopsy (Case 1, Case 2). More than 20 genes have been implicated in the various congenital myopathies to date. Mutations in the skeletal muscle ryanodine receptor (*RYR1*) gene are the most frequent genetic cause and have been associated with dominantly inherited CCD and subgroups of recessively inherited MmD, CNM and CFTD of which the first two (the “core myopathies” [19]) are the most common. Muscle imaging of the lower leg in *RYR1*-related core myopathies [16, 20], shows a distinct pattern characterized by prominent involvement of vasti, adductor magnus and sartorius in the thigh and of gastrocnemii and soleus in the leg, with relative sparing of the rectus femoris, adductor longus, gracilis and tibialis anterior (Table 2, Figure 3C, Figure 4A-B, Case 1). Although the pattern of selective involvement is more diffuse in recessive compared to dominantly inherited forms, it is similar throughout all forms of *RYR1*-related myopathies and may be more consistent than associated histopathological features (Case 1) [21]. Recent whole body MRI studies in *RYR1*-related myopathies [22] (Figure 5) have demonstrated additional involvement of shoulder, neck, masticator and paravertebral muscles, the latter also a feature in the dominantly inherited *RYR1*-related malignant hyperthermia susceptibility trait, an allelic pharmacogenetic reaction to certain general anesthetics and muscle relaxants [23]. Muscle imaging may aid the differential diagnosis

between *RYR1*-related myopathies and other conditions with overlapping clinical and histopathological features, in particular those due to mutations in the *SEPNI*, the collagen 6 (*COL6*) and *DNM2* genes. Recessive mutations in *SEPNI* are the second most common cause of core myopathies, in particular of the MmD variant [24]. Clinically, *SEPNI*-related MmD is characterized by early-onset scoliosis with spinal rigidity and respiratory impairment, but in contrast to recessive *RYR1*-related MmD, extraocular muscles are typically spared. Muscle MRI involvement in *SEPNI*-related myopathies (Table 2, Figure 3E) has been documented in several series [22, 25]: In addition to the striking axial involvement visible on whole body MRI, patients with *SEPNI*-related MmD often show marked wasting of the inner thigh (“bracket-like” appearance) [22, 25]. Within the thigh, the sartorius and semimembranosus muscles are most consistently involved; in cases where the lower leg is affected, gastrocnemius medialis and soleus tend to be more prominently affected (Table 2) [25]. Early and prominent sartorius involvement (Figure 3H) distinguishes *SEPNI*-related myopathies from other genetic causes of the rigid spine syndrome (RSS), in particular mutations in Lamin A/C (*LMNA*), the *COL6* genes and acid maltase deficiency, as well as *RYR1*-related myopathies, where this feature usually occurs later in the disease course [26].

Centronuclear Myopathy (CNM) is due to X-linked recessive mutations in *MTM1* encoding myotubularin [“X-linked myotubular myopathy (XLMTM)”] [27], autosomal-dominant mutations in *DNM2* encoding dynamin2 [28] and the *BINI* gene encoding amphiphysin 2 [29], autosomal-recessive mutations in *BINI* [30], *RYR1* [31], and *TTN* encoding titin [32], and rarer genetic backgrounds [33-35]. Clinically, the associated muscle weakness is highly variable but, with the exception of *TTN*-related CNM, extraocular muscles are consistently involved. Muscle imaging studies in XLMTM have been mainly performed in the rare subset of more mildly affected males and manifesting female carriers, also often featuring “necklace fibres” in addition to diagnostic central nuclei on muscle biopsy [36]. In this group, the

reported pattern of selective muscle involvement (Table 2) shows some similarities to those reported in *RYR1*-related myopathies, in particular with regards to relative sparing of rectus femoris, and gracilis within an otherwise more diffusely affected thigh, which is of interest considering some shared pathogenic mechanisms between the two conditions [37]. Dominantly inherited *DNM2*-related CNM [28] is usually a much milder condition with the exception of rare cases associated with *de novo* inheritance [38]. Several studies have reported muscle MRI findings in *DNM2*-related CNM, suggesting some consistent and diagnostically useful features but also considerable variability, probably reflecting marked age differences in the cohorts studied [20, 39-41] (Table 2): In contrast to all other forms of CNM, distal weakness is prominent, and reflected in early and severe involvement of the tibialis anterior, the gastrocnemius (medialis) and the soleus. The thigh muscles are much later and less severely affected, with initial involvement of the posterior and later and milder involvement of the anterior compartment. Although not universally present, preferential involvement of rectus femoris and adductor longus [40] represents a mirror image of the pattern seen in *RYR1*-related myopathies and may support the distinction from *RYR1*-related CNM in individual cases. Marked axial and masticator involvement are additional supportive features on whole muscle MRI [22]. There are currently no larger series regarding imaging findings in *TTN*-related CNM and it remains to be seen if the pattern is similar to that seen in other *TTN*-related myopathies, demonstrating early hamstring involvement, in particular of the semitendinosus (Figure 3A).

Nemaline myopathy (NM) is one of the more common congenital myopathies [42-44] and characterized by numerous nemaline rods on muscle biopsy. To date, 10 genes have been linked to this disorder, including *ACTA1*, *NEB*, *TPM3*, *TPM2*, *CFL2*, *TNNT1*, *LMOD3*, *KBTBD13*, *KLHL40*, and *KLHL41* [45-47], the majority of those implicated in thin filament assembly and interaction. NM is associated with highly variable degrees of muscle weakness

[44, 46, 48, 49]. Bulbar and respiratory impairment are common in many genetic forms but extraocular muscle involvement is not a typical feature, except in those due to mutations in the *KLHL* genes.

Recessive mutations in *NEB* and (*de novo*) dominant mutations in *ACTA1* are the most common causes of NM. The pattern of selective muscle involvement in *NEB*-related NM (Table 2) has been reported in one small series [50] and is characterized in mild cases by complete sparing of the thigh muscles and selective involvement of the tibialis anterior and soleus (Case 3 and 4). In moderate cases, there may be predominant involvement of rectus femoris, vastus lateralis and hamstring muscles and diffuse involvement of anterior compartment and soleus. Muscle imaging in *ACTA1*-related myopathies has been reported in isolated cases and families with variable clinico-pathological and radiological features [50-52]. Whole body MRI performed in one family with a *TPM2*-related myopathy suggested prominent rectus femoris, vastus lateralis and semimembranosus involvement in the thigh, and prominent soleus involvement in the leg [53].

Congenital muscular dystrophies

The congenital muscular dystrophies (CMDs) are a genetically heterogeneous group of conditions characterized by variable but often profound weakness, moderate to marked CK elevations and dystrophic features on muscle biopsy (for review, [54]). In contrast to the congenital myopathies, immunohistochemical studies demonstrating the reduction or absence of specific proteins are diagnostically more important than structural abnormalities on muscle biopsy. CMDs are most commonly caused by mutations in genes encoding for basal lamina and extracellular matrix components, in particular recessive mutations in the *LAMA* gene encoding laminin $\alpha 2$, genes involved in glycosylation of α -dystroglycan (the “dystroglycanopathies”), and the *COL6* and *LMNA* genes. Because of the profound weakness,

Muscle imaging and myopathology

muscles are often too diffusely involved to still exhibit a diagnostically relevant pattern, or may exhibit a “negative pattern” with the very few muscles spared providing a diagnostic clue. As there is often associated CNS involvement, brain imaging is at least as diagnostically relevant as muscle imaging, and may reveal characteristic abnormalities such as the typical white matter changes in *LAMA*-associated CMD, or a wide range of structural brain abnormalities in the dystroglycanopathies.

Muscle imaging has been most widely performed in forms of CMD due to recessive and dominant mutations in one of the 3 *COL6* genes [22, 55-57], a group of conditions where it may also be diagnostically most helpful, considering the often significant clinico-pathological overlap with the structural congenital myopathies (see above). At the more severe end of the spectrum, typically recessively inherited Ullrich congenital muscular dystrophy (UCMD) is characterized by usually marked weakness, proximal joint contractures in the context of distal laxity, and early respiratory impairment. Patients with the allelic and typically dominantly inherited Bethlem myopathy (BM) are usually more mildly affected, with a different contractural pattern and less severe respiratory impairment. In addition to variable myopathic or dystrophic changes on muscle biopsy, UCMD may show diagnostic reduction of collagen VI but this can be subtle or even normal; particularly in the latter cases muscle imaging may provide additional clues. Although some relative sparing (for example, of the rectus femoris, sartorius and gracilis within the thigh) (Table 2) has been reported, in the *COL6*-related myopathies the internal appearance of muscle on MRI (in particular hyperintense alternating normo- or hypointense areas) is diagnostically more important than the overall pattern of selectivity: This appearance has been described in many different ways (“tigroid” pattern, “rolled-cake” appearance), but essentially reflects a regularly structured co-existence of normal and abnormal tissue within the same muscle (Figure 3I). A similar pattern may be observed in isolated muscles in other neuromuscular disorders, for example *DYSF* and

CAPN3-related LGMD (see below), but is usually not as extensive as in the collagenopathies where a wide range of muscles – rectus femoris, vastus, soleus, gastrocnemius, triceps, deltoid – may be affected.

Muscle imaging in the other CMDs has been performed in milder forms of CMD due to *LAMA* mutations, demonstrating prominent posterior and medial involvement (mainly concerning the adductor magnus) within the thigh, and soleus muscle involvement in the lower legs. The pattern in milder cases of *LMNA*-related CMD shows a continuum with other laminopathies, particular in the lower limbs (see below), whereas in more severe cases, whole body MRI may be required to demonstrate residual sparing of forearm, psoas, masticator and tongue muscles as a diagnostic clue [22]. Although not specific, marked lipodystrophy is another diagnostic feature in laminopathies. The only form of dystroglycanopathy where muscle imaging has been more widely performed are those due mutations in the *FKRP* gene with considerable overlap with the LGMD2I spectrum (see below).

Dystrophinopathies

Although less relevant from a diagnostic perspective, there is a rapidly increasing body of literature concerning muscle MR imaging in Duchenne muscular dystrophy [58-68], Becker muscular dystrophy [69-72], and carriers of these conditions [73], with the main aim to delineate the timely and spatial pattern of selective muscle involvement more accurately and to quantify intramuscular fatty changes more reproducibly as a biomarker [60, 71]. The pattern of selective muscle involvement identified through these studies (Table 2) shows both variability and consistency in boys with DMD: Within the thigh, adductor magnus is involved early followed by vasti, rectus and biceps femoris, whereas other hamstring muscles become affected later and gracilis, adductor longus and (less frequently) sartorius may remain spared well into the disease course [58, 63]. Within the lower leg, soleus [64, 65] and gastrocnemius

[65] are the earliest muscles to be involved followed by the peroneal group. The pattern in BMD [69, 70] and manifesting DMD carriers [73] is similar but less severe and less progressive. Muscle MR imaging findings have been correlated with histological features [65], function and strength in DMD [66-68], and have also been used in the dystrophinopathies to assess the effects of exercise [62] and steroid treatment [59]. With an increasing number of therapies targeting the dystrophinopathies currently being developed, it is likely that in particular novel muscle imaging techniques will become increasingly relevant for natural history studies and to assess the effects of therapeutic interventions.

Emerinopathies and laminopathies

Emery-Dreifuss muscular dystrophy (EDMD) has been attributed to X-linked recessive mutations in the Emerin (*EDMD*) (*EDMD1*) and autosomal-dominant mutations in the *LMNA* gene (*EDMD2*) and is characterized by marked spinal rigidity, a scapuloperoneal distribution of weakness and often severe cardiac involvement. CK may be normal or mildly to moderately elevated. Muscle biopsy findings are variable, ranging from mild myopathic changes to complete fatty replacement of muscle; additional inflammatory changes may be prominent. Muscle imaging studies have been performed more frequently in the autosomal-dominant than the rarer X-linked recessive form [22, 74-76], and may help to distinguish these conditions from other genetic forms of the RSS (see above) [76, 77]. The typical pattern in *LMNA*-associated EDMD (Table 2) is characterized by prominent involvement of the lower leg, with marked affectation of the soleus and the gastrocnemius medialis compared to the gastrocnemius lateralis muscle [74]. In the less involved thigh, the adductors, vasti, semimembranosus and the long head of the biceps femoris are typically affected. Studies comparing the X-linked recessive and the autosomal-dominant forms directly suggest that the typical lower leg involvement may be present earlier in *EDMD2* [74], whereas at a later stage

EDMD1 may show a degree of peroneal involvement not present in EDMD2 [75]. A similar pattern varying in severity is seen in the wide group of other *LMNA*-related disorders (the “laminopathies” [78]), including bona fide neuromuscular phenotypes such as LGMD1B but also multisystemic manifestations including familial partial lipodystrophies and predominantly cardiac phenotypes [79, 80].

Limb girdle muscular dystrophies

The limb girdle muscular dystrophies (LGMDs) (for review, [81]) are a genetically heterogeneous group of conditions associated with both recessive and dominant inheritance, characterized clinically by proximal weakness often pronounced in the hip girdle, sparing of facial and extraocular muscles, and variable cardiorespiratory involvement.

In addition to non-specific dystrophic features on muscle biopsy, inflammatory changes may be prominent, occasionally giving rise to diagnostic confusion. Specific immunohistochemical studies may help in delineating specific genetic forms but are not always unequivocally diagnostic; muscle imaging may provide additional useful information in such cases. In the group of recessive LGMDs (designated as LGMD2 with alphabetic lettering representing the specific genetic background), the most common forms (in order of ascending age of presentation) are LGMD2C-F due to mutations in the sarcoglycan genes, LGMD2A due to mutations in the calpain 3 (*CAPN3*) gene, LGMD2I due to mutations in the *FKRP* gene, LGMD2B due to mutations in the dysferlin (*DYS*) gene, and LGMD2L due to mutations in the more recently discovered anoctamin 5 (*ANO5*) gene. Muscle imaging studies in the sarcoglycanopathies are rare, but in contrast to LGMD2A, LGMD2B and LGMD2I where posterior compartment involvement is prominent, show more pronounced anterior compartment involvement [5] similar to the pattern observed in the dystrophinopathies (see above) .

Muscle imaging and myopathology

Selective muscle involvement in LGMD2A has been documented in several series [82-84] and may provide useful supportive diagnostic information in the 20% of patients where calpain 3 expression on Western blot is normal. Corresponding to clinical findings, there is marked posterior compartment involvement in the pelvis (in particular gluteus maximus), within the thigh (in particular biceps femoris, semimembranosus and adductor muscles) and in the lower leg (soleus and gastrocnemius medialis) (Table 2, Figure 3B). The pattern in LGMD2I (Table 2) [82, 85] is similar with comparable posterior compartment emphasis, but may show more prominent involvement of the vastus lateralis, less selective involvement in the posterior calf muscles and more pronounced sparing of the often hypertrophic tibialis posterior [82]. The Dixon technique has been used to quantify fat more accurately as an outcome measure in LGMD2I [85]. The pattern in LGMD2B [82, 84, 86-88] is probably more variable compared to LGMD2A and LGMD2I, with more variable anterior and posterior involvement in different patients [82]. A recent comprehensive study of 27 patients investigated by whole body MRI at different ages [87] suggests early and severe involvement of the hamstring muscles and the adductors in the thigh, and of the soleus and gastrocnemius muscle in the leg. Of note, clinically distinct dysferlinopathies – LGMD2B, Miyoshi muscular dystrophy (MMD1) and distal myopathy with anterior tibial onset – often share the same pattern of selective involvement on muscle imaging [88]. The more recently described LGMD2L secondary to recessive mutations in the *ANO5* gene shows some clinical overlap with the dysferlinopathies, in particular if the presentation is more distal, but is distinguished by later onset, and cardiac involvement in some patients. The pattern on muscle MRI [89] (Table 2) with early posterior compartment involvement (Figure 3E) shows considerable overlap with other recessively inherited forms of LGMD, and despite the suggestion of minor distinguishing features in some series [90, 91], may not be particularly helpful to inform the differential diagnosis. As in other LGMDs, inflammatory changes may precede the

emergence of fatty muscle infiltration [92]. Muscle MRI may also be useful to distinguish late-onset acid maltase deficiency, occasionally presenting as a phenocopy of recessive LGMDs but showing unusually severe and early adductor involvement (Figure 3D) [93].

Dominantly inherited LGMDs (designated as LGMD1 with alphabetic lettering representing the specific genetic background) account for only 10% of all forms of all LGMDs and show considerable overlap with other dominantly inherited disorders. LGMD1A due to dominant mutations in *MYOT* encoding myotilin is allelic to a form of myofibrillar myopathy (MFM) (see below). LGMD1B due to dominant mutations in lamin A/C (*LMNA*) is part of the spectrum of the laminopathies, and the muscle MRI pattern in these conditions [22, 75, 76, 79, 80] has been discussed above. LGMD1C due to dominant mutations in the *CAV3* gene encoding caveolin 3 is part of a spectrum of disorders (the “caveolinopathies”), comprising LGMD, rippling muscle disease (RMD), asymptomatic hyperCKaemia and a distal myopathy. There are only few anecdotal reports describing muscle imaging studies in these conditions, suggesting a posterior and medial pattern in the thigh, with early involvement of the rectus femoris that is unusual in the context of other LGMDs, or most neuromuscular disorders in general [94, 95].

Fascioscapulohumeral muscular dystrophy (FSHD)

Fascioscapulohumeral muscular dystrophy (FSHD) is the third most common muscular dystrophy and in more than 95% of cases caused by a contracted D4Z4 macrosatellite array on the subtelomeric region of chromosome 4q. The condition usually presents from adolescence with facial, shoulder, abdominal and limb girdle weakness but atypical presentations are recognized.

FSHD has been extensively studied by muscle imaging (including a number of whole body MRI studies) [96-108], mainly with the aim to accurately delineate the pattern and quality of

muscle involvement with a view to natural history studies and as an outcome measure for therapeutic interventions. The trapezius, serratus anterior and teres major are the earliest and most severely affected muscles, followed by pectoralis and latissimus dorsi, with consistent sparing of the spinati and subscapularis muscles [97, 107]. In the lower limb (Table 2), there is early and severe involvement of the hamstring muscles and the adductors within the thigh, and of the soleus, gastrocnemius medialis and tibialis anterior in the lower leg. The combination of tibialis anterior involvement and lack of rectus femoris sparing is unusual in the context of other neuromuscular disorders and may provide a diagnostic clue. Although there is often asymmetrical involvement [97, 98, 106] this may not be as pronounced as expected on clinical grounds. Muscle edema may be an early feature [96]. The combination of marked axial muscle involvement and a suggestive pattern of involvement in the shoulder girdle and limbs may present a diagnostic clue in patients presenting with isolated camptocormia rather than more typical features [101, 106]. Additional studies have correlated muscle MR imaging findings and muscle function and strength in patients with FSHD [98, 109]. As with other common muscular dystrophies, there are ongoing efforts to quantify fat infiltration as a biomarker for natural history studies and therapeutic interventions [100, 103]

Myotonic dystrophies

Myotonic dystrophy is due to a dominantly inherited unstable triplet repeat expansion in the *DMPK* gene encoding dystrophin myotonic-protein kinase (DM1), or, less frequently, an unstable intronic CCTG expansion in the *CNBP/ZNF9* gene (DM2). DM1 is the most common adult muscular dystrophy and characterized by muscle weakness and wasting, myotonia, and multisystem involvement variably affecting the brain, heart, lens and the endocrine system.

DM1 has been extensively studied by muscle imaging [84, 110-117]. Although myotonic dystrophy is usually a clinical diagnosis, patients presenting with mild or atypical features may enter the differential diagnosis of other neuromuscular disorders, and knowledge of the pattern of selective involvement on muscle imaging is therefore useful: In DM1, there is prominent anterior compartment involvement in the thigh and prominent posterior compartment involvement in the lower leg. There is a typical “semilunar” appearance within the vasti, reflective of perifemoral degeneration, and additional semitendinosus and semimembranosus involvement. In the lower leg, there is prominent soleus and gastrocnemius medialis involvement, but the tibialis anterior is also often affected. Changes in the tibialis anterior have also been specifically investigated with a view to their potential as a biomarker in DM1 [116-118]. In one comparative study the pattern of selective involvement in DM2 was considered similar, with the exception of more marked involvement of the erector spinae muscles [112, 119].

Myofibrillar myopathies

The myofibrillar myopathies (MFMs) (for review, [120]) are a group of genetically heterogeneous, late-onset disorders with often prominent distal weakness and variable multisystem involvement. The histopathological hallmarks are areas of focal myofibrillar disruption, desmin-positive inclusions on immunohistochemistry and ultrastructural abnormalities on electron microscopy. MFMs have been attributed to mutations in *DES* encoding desmin, *CRYAB* encoding α B-crystallin, *MYOT* encoding myotilin, *FLNC* encoding filamin C, *BAG3* encoding Bcl-2-associated atonogene 3, *ZASP* encoding Z-band alternatively spliced PDZ-containing protein and other genetic backgrounds [120]. There is likely to be further genetic heterogeneity as around half of all patients with MFMs remain currently genetically unresolved. MFMs have to be distinguished from other neuromuscular

disorders and from each other, and as often neither clinical nor pathological features are entirely specific, muscle imaging may be very useful to inform the differential diagnosis. A large muscle imaging series of 46 patients with different MFMs (mainly related to mutations in *DES* and *MYOT*) [121] suggests that there are two distinct pattern of muscle involvement, one common to *DES*- and *CRYAB*-related forms and the other shared between *MYOT*-, *FLNC*- and *ZASP*-related forms. *DES*-related MFM is characterized within the thigh by early semitendinosus, sartorius and gracilis involvement, the latter finding very unusual in other neuromuscular disorders (Table 2, Figure 3F). In the lower leg, there is prominent early peroneus involvement. The pattern in *MYOT*-related MFM represents almost a mirror image, with prominent involvement of the semimembranosus, biceps femoris and adductor magnus compared to the semitendinosus, and relative sparing of the gracilis and sartorius (Table 2, Figure 3G). In the lower leg, there is prominent involvement of the soleus, gastrocnemius medialis and also the tibialis anterior. A more recent whole body MRI study comparing *DES*- and *MYOT*-related MFM confirms above features and provides additional information regarding upper limb involvement [122, 123].

CONCLUSIONS AND OUTLOOK

Over the last two decades muscle imaging has become an established modality both for the diagnosis and monitoring of neuromuscular disorders. In future, the increasing use of whole body MRI protocols will further increase the diagnostic yield, in particular if combined with computational meta-analytical approaches that are likely to replace the currently prevailing and often subjective interpretation of lower limb muscle MRIs; these new techniques of automatic graphical representation are increasingly used in a diagnostic setting and have already provided the basis for some of the studies cited in the present review. Recent rapid

genetic advances will result in genetic testing performed increasingly early in the diagnostic process, however, muscle imaging is likely to retain its role, probably with a stronger emphasis on aiding genetic variant interpretation rather than informing the primary choice of gene(s) to be analysed. Whilst common neuromuscular disorders have been studied in larger series providing reliable information regarding the typical selective involvement, in particular novel and rare genetic neuromuscular disorders will require substantial collaborative multicentre efforts to establish diagnostic patterns. There is also a need for comparative studies, correlating for example muscle MRI with muscle US findings as a method that can be applied more readily and at less cost at the bedside. Lastly, novel quantitative muscle imaging techniques allowing reliable and reproducible muscle fat fraction assessments will result in the increasing use of muscle MR imaging in natural history studies and as an outcome measure in clinical trials.

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REFERENCES

- 1 Del Grande F, Carrino JA, Del Grande M, Mammen AL, Christopher Stine L. Magnetic resonance imaging of inflammatory myopathies. *Top Magn Reson Imaging* 2011; 22: 39-43
- 2 Kermarrec E, Demondion X, Khalil C, Le Thuc V, Boutry N, Cotten A. Ultrasound and magnetic resonance imaging of the peripheral nerves: current techniques, promising directions, and open issues. *Semin Musculoskelet Radiol* 2010; 14: 463-72
- 3 Pillen S, Arts IM, Zwarts MJ. Muscle ultrasound in neuromuscular disorders. *Muscle & nerve* 2008; 37: 679-93
- 4 Wattjes MP, Fischer D, Eds. *Neuromuscular Imaging*. New York: Springer. 2013
- 5 Wattjes MP, Kley RA, Fischer D. Neuromuscular imaging in inherited muscle diseases. *Eur Radiol* 2010; 20: 2447-60
- 6 Murphy WA, Totty WG, Carroll JE. MRI of normal and pathologic skeletal muscle. *AJR American journal of roentgenology* 1986; 146: 565-74
- 7 Fischer D, Bonati U, Wattjes MP. Recent developments in muscle imaging of neuromuscular disorders. *Current opinion in neurology* 2016:
- 8 Glover GH, Schneider E. Three-point Dixon technique for true water/fat decomposition with B0 inhomogeneity correction. *Magn Reson Med* 1991; 18: 371-83
- 9 Wren TA, Bluml S, Tseng-Ong L, Gilsanz V. Three-point technique of fat quantification of muscle tissue as a marker of disease progression in Duchenne muscular dystrophy: preliminary study. *AJR American journal of roentgenology* 2008; 190: W8-12
- 10 Grehl T, Muller K, Vorgerd M, Tegenthoff M, Malin JP, Zange J. Impaired aerobic glycolysis in muscle phosphofructokinase deficiency results in biphasic post-exercise phosphocreatine recovery in ³¹P magnetic resonance spectroscopy. *Neuromuscular disorders* : NMD 1998; 8: 480-8

- 11 Hsieh TJ, Jaw TS, Chuang HY, Jong YJ, Liu GC, Li CW. Muscle metabolism in Duchenne muscular dystrophy assessed by in vivo proton magnetic resonance spectroscopy. *J Comput Assist Tomogr* 2009; 33: 150-4
- 12 Lodi R, Muntoni F, Taylor J, Kumar S, Sewry CA, Blamire A, Styles P, Taylor DJ. Correlative MR imaging and ³¹P-MR spectroscopy study in sarcoglycan deficient limb girdle muscular dystrophy. *Neuromuscular disorders : NMD* 1997; 7: 505-11
- 13 Lodi R, Kemp GJ, Muntoni F, Thompson CH, Rae C, Taylor J, Styles P, Taylor DJ. Reduced cytosolic acidification during exercise suggests defective glycolytic activity in skeletal muscle of patients with Becker muscular dystrophy. An in vivo ³¹P magnetic resonance spectroscopy study. *Brain : a journal of neurology* 1999; 122 (Pt 1): 121-30
- 14 Nagel AM, Amarteifio E, Lehmann-Horn F, Jurkat-Rott K, Semmler W, Schad LR, Weber MA. 3 Tesla sodium inversion recovery magnetic resonance imaging allows for improved visualization of intracellular sodium content changes in muscular channelopathies. *Investigative radiology* 2011; 46: 759-66
- 15 Lamminen AE. Magnetic resonance imaging of primary skeletal muscle diseases: patterns of distribution and severity of involvement. *The British journal of radiology* 1990; 63: 946-50
- 16 Jungbluth H, Davis MR, Muller C, Counsell S, Allsop J, Chattopadhyay A, Messina S, Mercuri E, Laing NG, Sewry CA, Bydder G, Muntoni F. Magnetic resonance imaging of muscle in congenital myopathies associated with RYR1 mutations. *Neuromuscul Disord* 2004; 14: 785-90
- 17 Mercuri E, Pichiecchio A, Counsell S, Allsop J, Cini C, Jungbluth H, Uggetti C, Bydder G. A short protocol for muscle MRI in children with muscular dystrophies. *Eur J Paediatr Neurol* 2002; 6: 305-7

- 18 North KN, Wang CH, Clarke N, Jungbluth H, Vainzof M, Dowling JJ, Amburgey K, Quijano-Roy S, Beggs AH, Sewry C, Laing NG, Bonnemann CG. Approach to the diagnosis of congenital myopathies. *Neuromuscular disorders : NMD* 2013;
- 19 Jungbluth H, Sewry CA, Muntoni F. Core myopathies. *Seminars in pediatric neurology* 2011; 18: 239-49
- 20 Fischer D, Herasse M, Bitoun M, Barragan-Campos HM, Chiras J, Laforet P, Fardeau M, Eymard B, Guicheney P, Romero NB. Characterization of the muscle involvement in dynamin 2-related centronuclear myopathy. *Brain : a journal of neurology* 2006; 129: 1463-9
- 21 Jungbluth H, Muller CR, Halliger-Keller B, Brockington M, Brown SC, Feng L, Chattopadhyay A, Mercuri E, Manzur AY, Ferreira A, Laing NG, Davis MR, Roper HP, Dubowitz V, Bydder G, Sewry CA, Muntoni F. Autosomal recessive inheritance of RYR1 mutations in a congenital myopathy with cores. *Neurology* 2002; 59: 284-7
- 22 Quijano-Roy S, Avila-Smirnow D, Carlier RY. Whole body muscle MRI protocol: pattern recognition in early onset NM disorders. *Neuromuscular disorders : NMD* 2012; 22 Suppl 2: S68-84
- 23 Loseth S, Voermans NC, Torbergesen T, Lillis S, Jonsrud C, Lindal S, Kamsteeg EJ, Lammens M, Broman M, Dekomien G, Maddison P, Muntoni F, Sewry C, Radunovic A, de Visser M, Straub V, van Engelen B, Jungbluth H. A novel late-onset axial myopathy associated with mutations in the skeletal muscle ryanodine receptor (RYR1) gene. *Journal of neurology* 2013; 260: 1504-10
- 24 Ferreira A, Quijano-Roy S, Pichereau C, Moghadaszadeh B, Goemans N, Bonnemann C, Jungbluth H, Straub V, Villanova M, Leroy JP, Romero NB, Martin JJ, Muntoni F, Voit T, Estournet B, Richard P, Fardeau M, Guicheney P. Mutations of the selenoprotein N gene, which is implicated in rigid spine muscular dystrophy, cause the classical phenotype of

multiminicore disease: reassessing the nosology of early-onset myopathies. *Am J Hum Genet* 2002; 71: 739-49

25 Hankiewicz K, Carlier RY, Lazaro L, Linzoain J, Barnerias C, Gomez-Andres D, Avila-Smirnow D, Ferreira A, Estournet B, Guicheney P, Germain DP, Richard P, Bulacio S, Mompoin D, Quijano-Roy S. Whole-body muscle magnetic resonance imaging in SEPN1-related myopathy shows a homogeneous and recognizable pattern. *Muscle & nerve* 2015; 52: 728-35

26 Mercuri E, Talim B, Moghadaszadeh B, Petit N, Brockington M, Counsell S, Guicheney P, Muntoni F, Merlini L. Clinical and imaging findings in six cases of congenital muscular dystrophy with rigid spine syndrome linked to chromosome 1p (RSMD1). *Neuromuscul Disord* 2002; 12: 631-8

27 Laporte J, Hu LJ, Kretz C, Mandel JL, Kioschis P, Coy JF, Klauck SM, Poustka A, Dahl N. A gene mutated in X-linked myotubular myopathy defines a new putative tyrosine phosphatase family conserved in yeast. *Nat Genet* 1996; 13: 175-82

28 Bitoun M, Maugenre S, Jeannet PY, Lacene E, Ferrer X, Laforet P, Martin JJ, Laporte J, Lochmuller H, Beggs AH, Fardeau M, Eymard B, Romero NB, Guicheney P. Mutations in dynamin 2 cause dominant centronuclear myopathy. *Nat Genet* 2005; 37: 1207-9

29 Bohm J, Biancalana V, Malfatti E, Dondaine N, Koch C, Vasli N, Kress W, Strittmatter M, Taratuto AL, Gonorazky H, Laforet P, Maisonobe T, Olive M, Gonzalez-Mera L, Fardeau M, Carriere N, Clavelou P, Eymard B, Bitoun M, Rendu J, Faure J, Weis J, Mandel JL, Romero NB, Laporte J. Adult-onset autosomal dominant centronuclear myopathy due to BIN1 mutations. *Brain : a journal of neurology* 2014:

30 Nicot AS, Toussaint A, Tosch V, Kretz C, Wallgren-Pettersson C, Iwarsson E, Kingston H, Garnier JM, Biancalana V, Oldfors A, Mandel JL, Laporte J. Mutations in

amphiphysin 2 (BIN1) disrupt interaction with dynamin 2 and cause autosomal recessive centronuclear myopathy. *Nat Genet* 2007; 39: 1134-9

31 Wilmshurst JM, Lillis S, Zhou H, Pillay K, Henderson H, Kress W, Muller CR, Ndondo A, Cloke V, Cullup T, Bertini E, Boennemann C, Straub V, Quinlivan R, Dowling JJ, Al-Sarraj S, Treves S, Abbs S, Manzur AY, Sewry CA, Muntoni F, Jungbluth H. RYR1 mutations are a common cause of congenital myopathies with central nuclei. *Ann Neurol* 2010; 68: 717-26

32 Ceyhan-Birsoy O, Agrawal PB, Hidalgo C, Schmitz-Abe K, Dechene ET, Swanson LC, Soemedi R, Vasli N, Iannaccone ST, Shieh PB, Shur N, Dennison JM, Lawlor MW, Laporte J, Markianos K, Fairbrother WG, Granzier H, Beggs AH. Recessive truncating titin gene, TTN, mutations presenting as centronuclear myopathy. *Neurology* 2013:

33 Tosch V, Rohde HM, Tronchere H, Zanolati E, Monroy N, Kretz C, Dondaine N, Payrastre B, Mandel JL, Laporte J. A novel PtdIns3P and PtdIns(3,5)P₂ phosphatase with an inactivating variant in centronuclear myopathy. *Human molecular genetics* 2006; 15: 3098-106

34 Majczenko K, Davidson AE, Camelo-Piragua S, Agrawal PB, Manfready RA, Li X, Joshi S, Xu J, Peng W, Beggs AH, Li JZ, Burmeister M, Dowling JJ. Dominant mutation of CCDC78 in a unique congenital myopathy with prominent internal nuclei and atypical cores. *American journal of human genetics* 2012; 91: 365-71

35 Agrawal PB, Pierson CR, Joshi M, Liu X, Ravenscroft G, Moghadaszadeh B, Talabere T, Viola M, Swanson LC, Haliloglu G, Talim B, Yau KS, Allcock RJ, Laing NG, Perrella MA, Beggs AH. SPEG interacts with myotubularin, and its deficiency causes centronuclear myopathy with dilated cardiomyopathy. *American journal of human genetics* 2014; 95: 218-26

- 36 Bevilacqua JA, Bitoun M, Biancalana V, Oldfors A, Stoltenburg G, Claeys KG, Lacene E, Brochier G, Manere L, Laforet P, Eymard B, Guicheney P, Fardeau M, Romero NB. "Necklace" fibers, a new histological marker of late-onset MTM1-related centronuclear myopathy. *Acta Neuropathol* 2009; 117: 283-91
- 37 Jungbluth H, Gautel M. Pathogenic mechanisms in centronuclear myopathies. *Front Aging Neurosci* 2014; 6: 339
- 38 Bitoun M, Bevilacqua JA, Prudhon B, Maugenre S, Taratuto AL, Monges S, Lubieniecki F, Cances C, Uro-Coste E, Mayer M, Fardeau M, Romero NB, Guicheney P. Dynamin 2 mutations cause sporadic centronuclear myopathy with neonatal onset. *Annals of neurology* 2007; 62: 666-70
- 39 Susman RD, Quijano-Roy S, Yang N, Webster R, Clarke NF, Dowling J, Kennerson M, Nicholson G, Biancalana V, Ilkovski B, Flanigan KM, Arbuckle S, Malladi C, Robinson P, Vucic S, Mayer M, Romero NB, Urtizberea JA, Garcia-Bragado F, Guicheney P, Bitoun M, Carrier RY, North KN. Expanding the clinical, pathological and MRI phenotype of DNM2-related centronuclear myopathy. *Neuromuscul Disord* 2010; 20: 229-37
- 40 Schessl J, Medne L, Hu Y, Zou Y, Brown MJ, Huse JT, Torigian DA, Jungbluth H, Goebel HH, Bonnemann CG. MRI in DNM2-related centronuclear myopathy: evidence for highly selective muscle involvement. *Neuromuscul Disord* 2007; 17: 28-32
- 41 Catteruccia M, Fattori F, Codemo V, Ruggiero L, Maggi L, Tasca G, Fiorillo C, Pane M, Berardinelli A, Verardo M, Bragato C, Mora M, Morandi L, Bruno C, Santoro L, Pegoraro E, Mercuri E, Bertini E, D'Amico A. Centronuclear myopathy related to dynamin 2 mutations: clinical, morphological, muscle imaging and genetic features of an Italian cohort. *Neuromuscular disorders* : NMD 2013; 23: 229-38
- 42 Maggi L, Scoto M, Cirak S, Robb SA, Klein A, Lillis S, Cullup T, Feng L, Manzur AY, Sewry CA, Abbs S, Jungbluth H, Muntoni F. Congenital myopathies--clinical features

and frequency of individual subtypes diagnosed over a 5-year period in the United Kingdom.

Neuromuscular disorders : NMD 2013; 23: 195-205

43 Romero NB, Sandaradura SA, Clarke NF. Recent advances in nemaline myopathy.

Current opinion in neurology 2013; 26: 519-26

44 Witting N, Werlauff U, Duno M, Vissing J. Prevalence and phenotypes of congenital myopathy due to alpha-actin 1 gene mutations. Muscle & nerve 2015:

45 Nowak KJ, Ravenscroft G, Laing NG. Skeletal muscle alpha-actin diseases (actinopathies): pathology and mechanisms. Acta Neuropathol 2013; 125: 19-32

46 Romero NB, Sandaradura SA, Clarke NF. Recent advances in nemaline myopathy.

Current opinion in neurology 2013; 26: 519-26

47 Yuen M, Sandaradura SA, Dowling JJ, Kostyukova AS, Moroz N, Quinlan KG, Lehtokari VL, Ravenscroft G, Todd EJ, Ceyhan-Birsoy O, Gokhin DS, Maluenda J, Lek M, Nolent F, Pappas CT, Novak SM, D'Amico A, Malfatti E, Thomas BP, Gabriel SB, Gupta N, Daly MJ, Ilkovski B, Houweling PJ, Davidson AE, Swanson LC, Brownstein CA, Gupta VA, Medne L, Shannon P, Martin N, Bick DP, Flisberg A, Holmberg E, Van den Bergh P, Lapunzina P, Waddell LB, Sloboda DD, Bertini E, Chitayat D, Telfer WR, Laquerriere A, Gregorio CC, Ottenheijm CA, Bonnemann CG, Pelin K, Beggs AH, Hayashi YK, Romero NB, Laing NG, Nishino I, Wallgren-Pettersson C, Melki J, Fowler VM, MacArthur DG, North KN, Clarke NF. Leiomodin-3 dysfunction results in thin filament disorganization and nemaline myopathy. The Journal of clinical investigation 2015; 125: 456-7

48 Maggi L, Scoto M, Cirak S, Robb SA, Klein A, Lillis S, Cullup T, Feng L, Manzur AY, Sewry CA, Abbs S, Jungbluth H, Muntoni F. Congenital myopathies--clinical features and frequency of individual subtypes diagnosed over a 5-year period in the United Kingdom.

Neuromuscular disorders : NMD 2013; 23: 195-205

- 49 Ryan MM, Schnell C, Strickland CD, Shield LK, Morgan G, Iannaccone ST, Laing NG, Beggs AH, North KN. Nemaline myopathy: a clinical study of 143 cases. *Ann Neurol* 2001; 50: 312-20
- 50 Jungbluth H, Sewry CA, Counsell S, Allsop J, Chattopadhyay A, Mercuri E, North K, Laing N, Bydder G, Pelin K, Wallgren-Pettersson C, Muntoni F. Magnetic resonance imaging of muscle in nemaline myopathy. *Neuromuscul Disord* 2004; 14: 779-84
- 51 Zukosky K, Meilleur K, Traynor BJ, Dastgir J, Medne L, Devoto M, Collins J, Rooney J, Zou Y, Yang ML, Gibbs JR, Meier M, Stetefeld J, Finkel RS, Schessl J, Elman L, Felice K, Ferguson TA, Ceyhan-Birsoy O, Beggs AH, Tennekoon G, Johnson JO, Bonnemann CG. Association of a Novel ACTA1 Mutation With a Dominant Progressive Scapuloperoneal Myopathy in an Extended Family. *JAMA Neurol* 2015; 72: 689-98
- 52 Castiglioni C, Cassandrini D, Fattori F, Bellacchio E, D'Amico A, Alvarez K, Gejman R, Diaz J, Santorelli FM, Romero NB, Bertini E, Bevilacqua JA. Muscle magnetic resonance imaging and histopathology in ACTA1-related congenital nemaline myopathy. *Muscle & nerve* 2014; 50: 1011-6
- 53 Jarraya M, Quijano-Roy S, Monnier N, Behin A, Avila-Smirnov D, Romero NB, Allamand V, Richard P, Barois A, May A, Estournet B, Mercuri E, Carlier PG, Carlier RY. Whole-Body muscle MRI in a series of patients with congenital myopathy related to TPM2 gene mutations. *Neuromuscular disorders : NMD* 2012; 22 Suppl 2: S137-47
- 54 Bonnemann CG, Wang CH, Quijano-Roy S, Deconinck N, Bertini E, Ferreira A, Muntoni F, Sewry C, Beroud C, Mathews KD, Moore SA, Bellini J, Rutkowski A, North KN. Diagnostic approach to the congenital muscular dystrophies. *Neuromuscular disorders : NMD* 2014; 24: 289-311

- 55 Mercuri E, Lampe A, Allsop J, Knight R, Pane M, Kinali M, Bonnemann C, Flanigan K, Lapini I, Bushby K, Pepe G, Muntoni F. Muscle MRI in Ullrich congenital muscular dystrophy and Bethlem myopathy. *Neuromuscular disorders* : NMD 2005; 15: 303-10
- 56 Mercuri E, Cini C, Pichiecchio A, Allsop J, Counsell S, Zolkipli Z, Messina S, Kinali M, Brown SC, Jimenez C, Brockington M, Yuva Y, Sewry CA, Muntoni F. Muscle magnetic resonance imaging in patients with congenital muscular dystrophy and Ullrich phenotype. *Neuromuscular disorders* : NMD 2003; 13: 554-8
- 57 Mercuri E, Cini C, Counsell S, Allsop J, Zolkipli Z, Jungbluth H, Sewry C, Brown SC, Pepe G, Muntoni F. Muscle MRI findings in a three-generation family affected by Bethlem myopathy. *Eur J Paediatr Neurol* 2002; 6: 309-14
- 58 Kim HK, Laor T, Horn PS, Racadio JM, Wong B, Dardzinski BJ. T2 mapping in Duchenne muscular dystrophy: distribution of disease activity and correlation with clinical assessments. *Radiology* 2010; 255: 899-908
- 59 Kim HK, Laor T, Horn PS, Wong B. Quantitative assessment of the T2 relaxation time of the gluteus muscles in children with Duchenne muscular dystrophy: a comparative study before and after steroid treatment. *Korean J Radiol* 2010; 11: 304-11
- 60 Gaeta M, Messina S, Mileto A, Vita GL, Ascenti G, Vinci S, Bottari A, Vita G, Settineri N, Bruschetta D, Racchiusa S, Minutoli F. Muscle fat-fraction and mapping in Duchenne muscular dystrophy: evaluation of disease distribution and correlation with clinical assessments. Preliminary experience. *Skeletal radiology* 2012; 41: 955-61
- 61 Liu M, Chino N, Ishihara T. Muscle damage progression in Duchenne muscular dystrophy evaluated by a new quantitative computed tomography method. *Archives of physical medicine and rehabilitation* 1993; 74: 507-14

- 62 Garrood P, Hollingsworth KG, Eagle M, Aribisala BS, Birchall D, Bushby K, Straub V. MR imaging in Duchenne muscular dystrophy: quantification of T1-weighted signal, contrast uptake, and the effects of exercise. *J Magn Reson Imaging* 2009; 30: 1130-8
- 63 Li W, Zheng Y, Zhang W, Wang Z, Xiao J, Yuan Y. Progression and variation of fatty infiltration of the thigh muscles in Duchenne muscular dystrophy, a muscle magnetic resonance imaging study. *Neuromuscular disorders : NMD* 2015; 25: 375-80
- 64 Torriani M, Townsend E, Thomas BJ, Bredella MA, Ghomi RH, Tseng BS. Lower leg muscle involvement in Duchenne muscular dystrophy: an MR imaging and spectroscopy study. *Skeletal radiology* 2012; 41: 437-45
- 65 Kinali M, Arechavala-Gomez V, Cirak S, Glover A, Guglieri M, Feng L, Hollingsworth KG, Hunt D, Jungbluth H, Roper HP, Quinlivan RM, Gosalakal JA, Jayawant S, Nadeau A, Hughes-Carre L, Manzur AY, Mercuri E, Morgan JE, Straub V, Bushby K, Sewry C, Rutherford M, Muntoni F. Muscle histology vs MRI in Duchenne muscular dystrophy. *Neurology* 2011; 76: 346-53
- 66 Vohra RS, Lott D, Mathur S, Senesac C, Deol J, Germain S, Bendixen R, Forbes SC, Sweeney HL, Walter GA, Vandenborne K. Magnetic Resonance Assessment of Hypertrophic and Pseudo-Hypertrophic Changes in Lower Leg Muscles of Boys with Duchenne Muscular Dystrophy and Their Relationship to Functional Measurements. *PLoS One* 2015; 10: e0128915
- 67 Wokke BH, van den Bergen JC, Versluis MJ, Niks EH, Milles J, Webb AG, van Zwet EW, Aartsma-Rus A, Verschuuren JJ, Kan HE. Quantitative MRI and strength measurements in the assessment of muscle quality in Duchenne muscular dystrophy. *Neuromuscular disorders : NMD* 2014; 24: 409-16

- 68 Ponrartana S, Ramos-Platt L, Wren TA, Hu HH, Perkins TG, Chia JM, Gilsanz V. Effectiveness of diffusion tensor imaging in assessing disease severity in Duchenne muscular dystrophy: preliminary study. *Pediatric radiology* 2015; 45: 582-9
- 69 Faridian-Aragh N, Wagner KR, Leung DG, Carrino JA. Magnetic resonance imaging phenotyping of Becker muscular dystrophy. *Muscle & nerve* 2014; 50: 962-7
- 70 Tasca G, Iannaccone E, Monforte M, Masciullo M, Bianco F, Laschena F, Ottaviani P, Pelliccioni M, Pane M, Mercuri E, Ricci E. Muscle MRI in Becker muscular dystrophy. *Neuromuscular disorders : NMD* 2012; 22 Suppl 2: S100-6
- 71 Loughran T, Higgins DM, McCallum M, Coombs A, Straub V, Hollingsworth KG. Improving highly accelerated fat fraction measurements for clinical trials in muscular dystrophy: origin and quantitative effect of R2* changes. *Radiology* 2015; 275: 570-8
- 72 van den Bergen JC, Wokke BH, Janson AA, van Duinen SG, Hulsker MA, Ginjaar HB, van Deutekom JC, Aartsma-Rus A, Kan HE, Verschuuren JJ. Dystrophin levels and clinical severity in Becker muscular dystrophy patients. *Journal of neurology, neurosurgery, and psychiatry* 2014; 85: 747-53
- 73 Tasca G, Monforte M, Iannaccone E, Laschena F, Ottaviani P, Silvestri G, Masciullo M, Mirabella M, Servidei S, Ricci E. Muscle MRI in female carriers of dystrophinopathy. *Eur J Neurol* 2012; 19: 1256-60
- 74 Mercuri E, Counsell S, Allsop J, Jungbluth H, Kinali M, Bonne G, Schwartz K, Bydder G, Dubowitz V, Muntoni F. Selective muscle involvement on magnetic resonance imaging in autosomal dominant Emery-Dreifuss muscular dystrophy. *Neuropediatrics* 2002; 33: 10-4
- 75 Diaz-Manera J, Alejaldre A, Gonzalez L, Olive M, Gomez-Andres D, Muelas N, Vilchez JJ, Llauger J, Carbonell P, Marquez-Infante C, Fernandez-Torron R, Poza JJ, Lopez de Munain A, Gonzalez-Quereda L, Mirabet S, Clarimon J, Gallano P, Rojas-Garcia R,

Gallardo E, Illa I. Muscle imaging in muscle dystrophies produced by mutations in the EMD and LMNA genes. *Neuromuscular disorders* : NMD 2016; 26: 33-40

76 Gomez-Andres D, Dabaj I, Mompoin D, Hankiewicz K, Azzi V, Ioos C, Romero NB, Ben Yaou R, Bergounioux J, Bonne G, Richard P, Estournet B, Yves-Carlier R, Quijano-Roy S. Pediatric laminopathies: Whole-body magnetic resonance imaging fingerprint and comparison with Sepn1 myopathy. *Muscle & nerve* 2016; 54: 192-202

77 Mercuri E, Clements E, Offiah A, Pichiecchio A, Vasco G, Bianco F, Berardinelli A, Manzur A, Pane M, Messina S, Gualandi F, Ricci E, Rutherford M, Muntoni F. Muscle magnetic resonance imaging involvement in muscular dystrophies with rigidity of the spine. *Annals of neurology* 2010; 67: 201-8

78 Politano L, Carboni N, Madej-Pilarczyk A, Marchel M, Nigro G, Fidziaoska A, Opolski G, Hausmanowa-Petrusewicz I. Advances in basic and clinical research in laminopathies. *Acta myologica : myopathies and cardiomyopathies : official journal of the Mediterranean Society of Myology / edited by the Gaetano Conte Academy for the study of striated muscle diseases* 2013; 32: 18-22

79 Carboni N, Mura M, Marrosu G, Cocco E, Ahmad M, Solla E, Mateddu A, Maioli MA, Marini S, Nissardi V, Frau J, Mallarini G, Mercuro G, Marrosu MG. Muscle MRI findings in patients with an apparently exclusive cardiac phenotype due to a novel LMNA gene mutation. *Neuromuscular disorders* : NMD 2008; 18: 291-8

80 Carboni N, Mura M, Marrosu G, Cocco E, Marini S, Solla E, Mateddu A, Maioli MA, Piras R, Mallarini G, Mercuro G, Porcu M, Marrosu MG. Muscle imaging analogies in a cohort of patients with different clinical phenotypes caused by LMNA gene mutations. *Muscle & nerve* 2010; 41: 458-63

81 Vissing J. Limb girdle muscular dystrophies: classification, clinical spectrum and emerging therapies. *Current opinion in neurology* 2016:

- 82 Fischer D, Walter MC, Kesper K, Petersen JA, Aurino S, Nigro V, Kubisch C, Meindl T, Lochmuller H, Wilhelm K, Urbach H, Schroder R. Diagnostic value of muscle MRI in differentiating LGMD2I from other LGMDs. *Journal of neurology* 2005; 252: 538-47
- 83 Mercuri E, Bushby K, Ricci E, Birchall D, Pane M, Kinali M, Allsop J, Nigro V, Saenz A, Nascimbeni A, Fulizio L, Angelini C, Muntoni F. Muscle MRI findings in patients with limb girdle muscular dystrophy with calpain 3 deficiency (LGMD2A) and early contractures. *Neuromuscular disorders : NMD* 2005; 15: 164-71
- 84 Stramare R, Beltrame V, Dal Borgo R, Gallimberti L, Frigo AC, Pegoraro E, Angelini C, Rubaltelli L, Feltrin GP. MRI in the assessment of muscular pathology: a comparison between limb-girdle muscular dystrophies, hyaline body myopathies and myotonic dystrophies. *Radiol Med* 2010; 115: 585-99
- 85 Willis TA, Hollingsworth KG, Coombs A, Sveen ML, Andersen S, Stojkovic T, Eagle M, Mayhew A, de Sousa PL, Dewar L, Morrow JM, Sinclair CD, Thornton JS, Bushby K, Lochmuller H, Hanna MG, Hogrel JY, Carlier PG, Vissing J, Straub V. Quantitative muscle MRI as an assessment tool for monitoring disease progression in LGMD2I: a multicentre longitudinal study. *PLoS One* 2013; 8: e70993
- 86 Kesper K, Kornblum C, Reimann J, Lutterbey G, Schroder R, Wattjes MP. Pattern of skeletal muscle involvement in primary dysferlinopathies: a whole-body 3.0-T magnetic resonance imaging study. *Acta neurologica Scandinavica* 2009; 120: 111-8
- 87 Diaz J, Woudt L, Suazo L, Garrido C, Caviedes P, AM CA, Castiglioni C, Bevilacqua JA. Broadening the imaging phenotype of dysferlinopathy at different disease stages. *Muscle & nerve* 2016; 54: 203-10
- 88 Paradas C, Llauger J, Diaz-Manera J, Rojas-Garcia R, De Luna N, Iturriaga C, Marquez C, Uson M, Hankiewicz K, Gallardo E, Illa I. Redefining dysferlinopathy

phenotypes based on clinical findings and muscle imaging studies. *Neurology* 2010; 75: 316-23

89 Sarkozy A, Deschauer M, Carlier RY, Schrank B, Seeger J, Walter MC, Schoser B, Reilich P, Leturq F, Radunovic A, Behin A, Laforet P, Eymard B, Schreiber H, Hicks D, Vaidya SS, Glaser D, Carlier PG, Bushby K, Lochmuller H, Straub V. Muscle MRI findings in limb girdle muscular dystrophy type 2L. *Neuromuscular disorders : NMD* 2012; 22 Suppl 2: S122-9

90 Ten Dam L, van der Kooi AJ, Rovekamp F, Linssen WH, de Visser M. Comparing clinical data and muscle imaging of DYSF and ANO5 related muscular dystrophies. *Neuromuscular disorders : NMD* 2014; 24: 1097-102

91 Tasca G, Evila A, Pane M, Monforte M, Graziano A, Hackman P, Mercuri E, Udd B. Isolated semitendinosus involvement in the initial stages of limb-girdle muscular dystrophy 2L. *Neuromuscular disorders : NMD* 2014; 24: 1118-9

92 Mahjneh I, Bashir R, Kiuru-Enari S, Linssen W, Lamminen A, Visser M. Selective pattern of muscle involvement seen in distal muscular dystrophy associated with anoctamin 5 mutations: a follow-up muscle MRI study. *Neuromuscular disorders : NMD* 2012; 22 Suppl 2: S130-6

93 Dlamini N, Jan W, Norwood F, Sheehan J, Spahr R, Al-Sarraj S, Anthony Hulse J, Hughes D, Champion MP, Jungbluth H. Muscle MRI findings in siblings with juvenile-onset acid maltase deficiency (Pompe disease). *Neuromuscul Disord* 2008; 18: 408-9

94 Fischer D, Schroers A, Blumcke I, Urbach H, Zerres K, Mortier W, Vorgerd M, Schroder R. Consequences of a novel caveolin-3 mutation in a large German family. *Annals of neurology* 2003; 53: 233-41

- 95 Jacobi C, Ruscheweyh R, Vorgerd M, Weber MA, Storch-Hagenlocher B, Meinck HM. Rippling muscle disease: variable phenotype in a family with five afflicted members. *Muscle & nerve* 2010; 41: 128-32
- 96 Friedman SD, Poliachik SL, Carter GT, Budech CB, Bird TD, Shaw DW. The magnetic resonance imaging spectrum of facioscapulohumeral muscular dystrophy. *Muscle & nerve* 2012; 45: 500-6
- 97 Gerevini S, Scarlato M, Maggi L, Cava M, Caliendo G, Pasanisi B, Falini A, Previtali SC, Morandi L. Muscle MRI findings in facioscapulohumeral muscular dystrophy. *Eur Radiol* 2016; 26: 693-705
- 98 Iosa M, Mazza C, Frusciante R, Zok M, Aprile I, Ricci E, Cappozzo A. Mobility assessment of patients with facioscapulohumeral dystrophy. *Clinical biomechanics* 2007; 22: 1074-82
- 99 Janssen BH, Pillen S, Voet NB, Heerschap A, van Engelen BG, van Alfen N. Quantitative muscle ultrasound versus quantitative magnetic resonance imaging in facioscapulohumeral dystrophy. *Muscle & nerve* 2014; 50: 968-75
- 100 Janssen BH, Voet NB, Nabuurs CI, Kan HE, de Rooy JW, Geurts AC, Padberg GW, van Engelen BG, Heerschap A. Distinct disease phases in muscles of facioscapulohumeral dystrophy patients identified by MR detected fat infiltration. *PLoS One* 2014; 9: e85416
- 101 Jordan B, Eger K, Koesling S, Zierz S. Camptocormia phenotype of FSHD: a clinical and MRI study on six patients. *Journal of neurology* 2011; 258: 866-73
- 102 Kan HE, Klomp DW, Wohlgemuth M, van Loosbroek-Wagemans I, van Engelen BG, Padberg GW, Heerschap A. Only fat infiltrated muscles in resting lower leg of FSHD patients show disturbed energy metabolism. *NMR Biomed* 2010; 23: 563-8

- 103 Lareau-Trudel E, Le Troter A, Ghattas B, Pouget J, Attarian S, Bendahan D, Salort-Campana E. Muscle Quantitative MR Imaging and Clustering Analysis in Patients with Facioscapulohumeral Muscular Dystrophy Type 1. *PLoS One* 2015; 10: e0132717
- 104 Leung DG, Carrino JA, Wagner KR, Jacobs MA. Whole-body magnetic resonance imaging evaluation of facioscapulohumeral muscular dystrophy. *Muscle & nerve* 2015; 52: 512-20
- 105 Olsen DB, Gideon P, Jeppesen TD, Vissing J. Leg muscle involvement in facioscapulohumeral muscular dystrophy assessed by MRI. *Journal of neurology* 2006; 253: 1437-41
- 106 Rijken NH, van der Kooi EL, Hendriks JC, van Asseldonk RJ, Padberg GW, Geurts AC, van Engelen BG. Skeletal muscle imaging in facioscapulohumeral muscular dystrophy, pattern and asymmetry of individual muscle involvement. *Neuromuscular disorders : NMD* 2014; 24: 1087-96
- 107 Tasca G, Monforte M, Iannaccone E, Laschena F, Ottaviani P, Leoncini E, Boccia S, Galluzzi G, Pelliccioni M, Masciullo M, Frusciante R, Mercuri E, Ricci E. Upper girdle imaging in facioscapulohumeral muscular dystrophy. *PLoS One* 2014; 9: e100292
- 108 Tasca G, Monforte M, Ottaviani P, Pelliccioni M, Frusciante R, Laschena F, Ricci E. Magnetic Resonance Imaging in a large cohort of facioscapulohumeral muscular dystrophy patients: pattern refinement and implications for clinical trials. *Annals of neurology* 2016:
- 109 Regula JU, Jestaedt L, Jende F, Bartsch A, Meinck HM, Weber MA. Clinical Muscle Testing Compared with Whole-Body Magnetic Resonance Imaging in Facio-scapulo-humeral Muscular Dystrophy. *Clin Neuroradiol* 2015:
- 110 Castillo J, Pumar JM, Rodriguez JR, Prieto JM, Arrojo L, Martinez F, Noya M. Magnetic resonance imaging of muscles in myotonic dystrophy. *Eur J Radiol* 1993; 17: 141-4

- 111 Damian MS, Bachmann G, Herrmann D, Dorndorf W. Magnetic resonance imaging of muscle and brain in myotonic dystrophy. *Journal of neurology* 1993; 240: 8-12
- 112 Kornblum C, Lutterbey G, Bogdanow M, Kesper K, Schild H, Schroder R, Wattjes MP. Distinct neuromuscular phenotypes in myotonic dystrophy types 1 and 2 : a whole body highfield MRI study. *Journal of neurology* 2006; 253: 753-61
- 113 Schedel H, Reimers CD, Nagele M, Witt TN, Pongratz DE, Vogl T. Imaging techniques in myotonic dystrophy. A comparative study of ultrasound, computed tomography and magnetic resonance imaging of skeletal muscles. *Eur J Radiol* 1992; 15: 230-8
- 114 Franc DT, Muetzel RL, Robinson PR, Rodriguez CP, Dalton JC, Naughton CE, Mueller BA, Wozniak JR, Lim KO, Day JW. Cerebral and muscle MRI abnormalities in myotonic dystrophy. *Neuromuscular disorders : NMD* 2012; 22: 483-91
- 115 Hayashi K, Hamano T, Kawamura Y, Kimura H, Matsunaga A, Ikawa M, Yamamura O, Mutoh T, Higuchi I, Kuriyama M, Nakamoto Y. Muscle MRI of the Upper Extremity in the Myotonic Dystrophy Type 1. *European neurology* 2016; 76: 87-94
- 116 Hiba B, Richard N, Hebert LJ, Cote C, Nejjar M, Vial C, Bouhour F, Puymirat J, Janier M. Quantitative assessment of skeletal muscle degeneration in patients with myotonic dystrophy type 1 using MRI. *J Magn Reson Imaging* 2012; 35: 678-85
- 117 Meola G. MRI of tibialis anterior as "surrogate measure" in myotonic dystrophy type 1. *The Canadian journal of neurological sciences Le journal canadien des sciences neurologiques* 2011; 38: 10-1
- 118 Cote C, Hiba B, Hebert LJ, Vial C, Remec JF, Janier M, Puymirat J. MRI of tibialis anterior skeletal muscle in myotonic dystrophy type 1. *The Canadian journal of neurological sciences Le journal canadien des sciences neurologiques* 2011; 38: 112-8

- 119 Schneider-Gold C, Beer M, Kostler H, Buchner S, Sandstede J, Hahn D, Toyka KV. Cardiac and skeletal muscle involvement in myotonic dystrophy type 2 (DM2): a quantitative ³¹P-MRS and MRI study. *Muscle & nerve* 2004; 30: 636-44
- 120 Kley RA, Olivé M, Schröder R. New aspects of myofibrillar myopathies. *Curr Opin Neurol.* 2016; 29:628-34.
- 121 Fischer D, Kley RA, Strach K, Meyer C, Sommer T, Eger K, Rolfs A, Meyer W, Pou A, Pradas J, Heyer CM, Grossmann A, Huebner A, Kress W, Reimann J, Schroder R, Eymard B, Fardeau M, Udd B, Goldfarb L, Vorgerd M, Olive M. Distinct muscle imaging patterns in myofibrillar myopathies. *Neurology* 2008; 71: 758-65
- 122 Schramm N, Born C, Weckbach S, Reilich P, Walter MC, Reiser MF. Involvement patterns in myotilinopathy and desminopathy detected by a novel neuromuscular whole-body MRI protocol. *Eur Radiol* 2008; 18: 2922-36
- 123 Olive M, Odgerel Z, Martinez A, Poza JJ, Bragado FG, Zabalza RJ, Jerico I, Gonzalez-Mera L, Shatunov A, Lee HS, Armstrong J, Maravi E, Arroyo MR, Pascual-Calvet J, Navarro C, Paradas C, Huerta M, Marquez F, Rivas EG, Pou A, Ferrer I, Goldfarb LG. Clinical and myopathological evaluation of early- and late-onset subtypes of myofibrillar myopathy. *Neuromuscular disorders : NMD* 2011; 21: 533-42
- 124 Klein A, Jungbluth H, Clement E, Lillis S, Abbs S, Munot P, Pane M, Wraige E, Schara U, Straub V, Mercuri E, Muntoni F. Muscle MRI in congenital myopathies due to Ryanodine receptor type 1 (RYR1) gene mutations

FIGURE AND TABLE LEGENDS

FIGURE LEGENDS

Figure 1 Correlation between histopathological and muscle MRI features. Muscle biopsies from the vastus lateralis (VL) (**A**) and the rectus femoris (RF) (**C**), hematoxylin & eosin (H&E) stains, transverse sections from a Paediatric patient with a recessive *RYR1*-related myopathy. Muscle magnetic resonance imaging (MRI) from the same patient, transverse section from the proximal thigh, T1-weighted images (**B**). Muscle biopsy from the vastus lateralis shows marked increases in fat and connective tissue, reflected in increased signal within the same muscle on T1-weighted MRI from the thigh. Muscle biopsy from the rectus femoris shows increased fibre size variability but less increase in connective tissue and fat compared to the vastus lateralis, reflected in relative sparing of the same muscle on T1-weighted MRI from the thigh.

Figure 2 Normal muscle anatomy in the lower limb. Schematic representation of normal muscle anatomy and T1-weighted muscle MR images from a normal individual, mid-thigh (**A**) and mid-calf (**B**) level. VL = vastus lateralis, VI = vastus intermedius, RF = rectus femoris, VM = vastus medialis, S = sartorius, G = gracilis, AM = adductor magnus, Sm = semimembranosus, St = semitendinosus, BL = biceps femoris, long head, AT = tibialis anterior, EDL = extensor digitorum longus, PG = peroneal group, Gl = gastrocnemius lateralis, Gm = gastrocnemius medialis, So = Soleus, TP = tibialis posterior.

Figure 3 Patterns of selective involvement in different genetic neuromuscular disorders.

T1-weighted images, transverse sections at the mid-thigh and mid-calf level from patients

with (A) *TTN*-related myopathy, (B) *CAPN3*-related LGMD2A, (C) *RYR1*-related congenital myopathy, (D) acid maltase deficiency, (E) *ANO5*-related LGMD2L, myofibrillar myopathies due to mutations in the (F) *DES* and (G) *MYOT* genes, (H) rigid spine syndrome (RSS) secondary to recessive *SEPN1* mutations, and (I) Bethlem myopathy secondary to dominant mutations in the *COL6* gene. For more detailed descriptions see main text. *Figure courtesy of Professor Volker Straub, Newcastle, United Kingdom.*

Figure 4 Muscle imaging and histopathological features from illustrative case studies.

T1-weighted muscle MR images from the thigh, (A) from a patient with a dominantly inherited and (B) Case 1 with a recessively inherited *RYR1*-related myopathy. The (C) muscle biopsy from Case 1 (NADH-TR, transverse section) showed only non-specific features, and *RYR1* testing was only performed because of the evocative muscle MRI pattern. (D) T1-weighted muscle MR images from the thigh from Case 2 with a recessively inherited *MYH2*-related myopathy. Despite a similar (E) muscle biopsy appearance with marked type 1 predominance and some unevenness of stain, the muscle MRI did not support *RYR1* involvement. T1-weighted muscle MR images from the lower leg in two sisters (older sister Case 3 (F), younger sister Case 4 (G)) with an unresolved congenital myopathy. The family refused a diagnostic muscle biopsy but, in the given clinical context, *NEB*-related nemaline myopathy was strongly suggested by marked tibialis anterior and soleus involvement, and was subsequently confirmed on specific genetic testing. Note intrafamilial consistency with almost identical muscle MRI findings in the two sisters.

Figure 5 Whole body MRI from a 16-year-old female with a *RYR1*-weighted myopathy and predominant proximal weakness.

Muscle imaging and myopathology

Selection of axial T1-weighted images from the head/face (A-D), shoulder girdle, arms and trunk (E-I), pelvis (J), thighs (K-N), legs (O-Q) and feet (R). In the face, the temporal muscles (A, *) and masseter (C, ++) are atrophic whereas lateral and medial pterygoid muscles are relatively better preserved. Muscles of the scapular girdle (E) are all atrophic and largely replaced by fat; the most affected muscles are the pectoralis major (white arrow) and the subscapularis (double short white arrowheads). The biceps brachialis (F) as well as the lumbar erector (H, I) and psoas/iliac (I-K, sinuous arrow) muscles are slightly atrophic and infiltrated with fat. In the thighs (L-N), there is diffuse involvement with relative sparing of the rectus femoris, sartorius, gracilis and adductor longus. In the lower legs and feet (O-R), there is diffuse involvement of the anterior compartment, soleus and medial gastrocnemius proximally, with the peroneal group more prominently involved distally. *Figure courtesy of Professor Robert Carlier, Paris, France.*

TABLE LEGENDS

Table 1 Correlation of histopathological abnormalities on muscle biopsy and muscle magnetic resonance imaging findings. SI = signal intensity; T1W = T1-weighted image; T2W = T2-weighted image; STIR = short tau inversion recovery

Table 2 Muscle involvement in selected inherited neuromuscular disorders.

Reported patterns of selective muscle involvement in the congenital myopathies (CM), congenital muscular dystrophies (CMD), Duchenne muscular dystrophy (DMD), Emery-Dreifuss muscular dystrophy (EDMD), limb girdle muscular dystrophies (LGMD), fascioscapulohumeral dystrophy (FSHD), myotonic dystrophy (DM1) and myofibrillar myopathies (MFM). Black boxes indicate muscle groups that are involved early and/or

Muscle imaging and myopathology

severely in the disease course, empty boxes indicate muscle groups that are consistently spared until late into the disease course, and shaded boxes indicate muscle groups that are involved later in the disease course or where there is variable involvement. VL = vastus lateralis, VI = vastus intermedius, RF = rectus femoris, VM = vastus medialis, Sa = sartorius, Gr = gracilis, AM = adductor magnus, Sm = semimembranosus, St = semitendinosus, BL = biceps femoris, long head, AT = tibialis anterior, EDL = extensor digitorum longus, PG = peroneal group, TP = tibialis posterior Gl = gastrocnemius lateralis, Gm = gastrocnemius medialis, So = Soleus.

Figure 1

Figure 2

Figure 3

Figure 4

SUPPLEMENTAL INFORMATION - CASE STUDIES

CASE 1 – Muscle MRI informing the choice of genetic testing

This 19-year-old lady (reported in [21]) presented with signs and symptoms of proximal weakness and a non-progressive mild scoliosis from the age of 4 years. She was the only child of a consanguineous Caucasian couple and there was no preceding family history of neuromuscular disorders or adverse reactions to general anaesthesia. Perinatal history and early developmental milestones had been normal.

On examination there was mild facial weakness but no ptosis and no extraocular muscle involvement. She had mild to moderate axial and proximal weakness pronounced in the hip girdle with an exaggerated lumbar lordosis. There was a mild scoliosis and bilateral Achilles tendon tightness but no other contractures.

Her further course remained stable. She remained fully ambulant but occasionally complaint about exertional myalgia. There were no bulbar or respiratory symptoms. Investigations included a normal CK at 151 IU/l. She had 3 muscle biopsies which were all considered myopathic but without suggesting a specific diagnosis. The most recent muscle biopsy (Figure 4C) performed at 15 years of age showed increased variability in fibre size and an increase in internalized nuclei, marked type 1 predominance and a few cores although those were not prominent. Muscle MRI (Figure 4B) demonstrated increased signal intensity within the adductor magnus, vasti and sartorius, with relative sparing of the rectus femoris, gracilis and hamstring muscles. Because of the striking similarities between the muscle MRI scan from the patient and the MRI scan from a patient with an already genetically resolved *RYR1*-related myopathy (Figure 4A), Sanger sequencing of *RYR1* mutational hotspots was initiated;

this revealed homozygosity for a C-terminal *RYR1* missense mutation (c.14545G>A; p.Val4849Ile), confirming the diagnosis of a recessive *RYR1*-related myopathy.

CASE 2 – Muscle MRI aiding genetic variant interpretation

This 14-year-old girl presented with mild motor developmental delay following an uneventful pregnancy. She was the 4th child of a consanguineous Asian couple and there was no preceding family history of neuromuscular disorders. She failed to thrive from early on in life despite adequate calorie intake in the absence of overt chewing or swallowing difficulties.

On examination there was mild facial weakness and ptosis with almost complete ophthalmoplegia. Weakness was moderate in axial muscles and in the shoulder girdle, and mild in the hip girdle.

Her further course remained stable in functional terms but she continued to complain about easy fatigability and myalgia. Ongoing failure to thrive required gastrostomy insertion resulting in improved weight gain.

Investigations included a normal CK. A muscle biopsy (Figure 4E) performed early in life had shown increased variability in fibre size and marked type 1 predominance with some unevenness of stain. *RYR1* sequencing prompted by the suspicion of a recessive *RYR1*-related myopathy revealed a heterozygous variant (c.5360C>G; p.Pro1787Leu) of uncertain significance but probably a polymorphism. Muscle MRI (Figure 4D) showed an almost normal appearance in the thigh and the lower leg, in contrast to most *RYR1*-related myopathies where at this stage at least some involvement of vasti and adductor magnus would be expected. Further testing through a congenital myopathy next generation sequencing panel revealed a homozygous mutation (*MYH2*) gene (c.2347C>T; p.Arg783Ter) in the myosin heavy chain 2 gene. She also carried a heterozygous mutation (c.1315C>T;

p.Arg439Ter) in the *SEPNI* gene which was not considered causative considering that the heterozygous carrier parent was asymptomatic, in keeping with the consistently recessive inheritance of *SEPNI*-related myopathies. Moreover, muscle MRI did not even show subtle features suggestive of a *SEPNI*-related myopathy (i.e. early sartorius involvement).

CASE 3 and 4 – Muscle MRI substituting for muscle biopsy

These 14- and 12-year-old sisters presented from early childhood with easy fatigability, a limited walking distance and difficulties running with frequent falls. Perinatal history and early developmental milestones were within normal limits. There was no history of bulbar or respiratory problems.

On examination, there was moderate facial weakness with non-fatiguable ptosis but no extraocular muscle involvement. In both sisters, there was moderate axial and proximal weakness with additional distal involvement pronounced in ankle dorsiflexion.

The further course remained stable in terms of functional abilities but both sisters developed a scoliosis and symptoms of nocturnal hypoventilation, requiring a thoracic brace and non-invasive night-time ventilation, respectively.

Investigations included a normal CK (161 IU/l) and normal EMG/NCS. The family were reluctant to consider a muscle biopsy because of its invasive nature. Muscle MRI showed an identical pattern in both sisters (Figure 4G and 4C), characterized by predominant involvement of the tibialis anterior, in the context of clinical features suggestive of *NEB*-related nemaline myopathy. Subsequent genetic testing through a next generation sequencing congenital myopathy panel revealed a homozygous *NEB* mutation (c.24178_24181dupGTCA; p.Lys8061fs) cosegregating with the disease phenotype.