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Muscle magnetic resonance imaging of a large cohort of distal hereditary motor neuropathies reveals characteristic features useful for diagnosis

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ABSTRACT

Distal motor neuropathies (dHMN) are an heterogenous group of diseases characterized by progressive muscle weakness affecting predominantly the distal muscles of the lower and upper limbs. Our aim was to study the imaging features and pattern of muscle involvement in muscle magnetic resonance imaging (MRI) in dHMN patients of suspected genetic origin (dHMN). We conducted a retrospective study collecting clinical, genetic and muscle imaging data. Muscle MRI included T1-weighted and T2 weighted Short Tau Inversion Recovery images (STIR-T2w) sequences. Muscle replacement by fat was quantified using the Mercuri score. Identification of selective patterns of involvement was performed using hierarchical clustering. Eighty-four patients with diagnosis of dHMN were studied. Fat replacement was predominant in the distal lower leg muscles (82/84 cases), although also affected thigh and pelvis muscles. Asymmetric involvement was present in 29% of patients. The superficial posterior compartment of the leg, including the soleus and gastrocnemius muscles, was the most affected area (77/84). We observed a reticular pattern of fatty replacement progressing towards what is commonly known as "muscle islands" in 79.8%. Hyperintensities in STIR-T2w were observed in 78.6% patients mainly in distal leg muscles. Besides features common to all individuals, we identified and describe a pattern of muscle

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fat replacement characteristic of *BICD2, HSPB1* and *DYNC1H1* patients. We conclude that muscle MRI of patients with suspected dHMN reveals common features helpful in diagnosis process.

1. Introduction

Distal hereditary motor neuropathies (dHMNs) are a highly heterogeneous group of rare diseases caused by degeneration of the motor nerves [1,2]. dHMNs are clinically characterized by slowly progressive symmetric distal muscle weakness and atrophy with minimal or no sensory involvement. Weakness affects predominantly the lower limbs although upper limb predominance has also been described [2]. The phenotype includes other less frequent clinical features such as pyramidal signs, tremor, vocal cord paralysis or respiratory dysfunction [3]. Disease onset is typically during childhood, but some patients might start later during the adulthood [4]. dHMNs includes dominant, recessive and X-linked diseases although a significant proportion of cases show a sporadic presentation. The implementation of next generation sequencing (NGS) has provided a genetic diagnosis to many patients and has increased the list of genes causing dHMNs that includes more than 30 genes. However, only 15 to 32% of patients with a syndromic diagnosis of dHMN have a confirmed genetic diagnosis suggesting, that there are still new genes causing the disease to be discovered [5,6,7,8]. Moreover, molecular definition by NGS has expanded the genotype-phenotype correlation spectrum. For example, pathogenic variants in the *HSPB1* and *HSPB8* genes are associated with the classical length-dependent motor neuropathy phenotype affecting predominantly the lower limbs, but they can also produce a myopathy complicating the clinical identification [9–11]. In contrast, mutations in *BSCL2 or GARS* gene are associated with a wider phenotype spectrum including also upper limb distal weakness and atrophy of the intrinsic muscles of the hand and/or pyramidal signs [12,13]. Vocal cord paralysis secondary to recurrent laryngeal nerve involvement is a feature of dHMN in patients with pathogenic variants in *DCTN1, SLC5A7* or *TRPV4* [14,15]. In other cases, motor neuropathy is the leading feature, but associated to other neurologic symptoms such as deafness, ataxia or optic atrophy [7]. These clinical features can be useful for guiding both the syndromic and genetic diagnosis towards dHMNs, but most of the patients present with isolated distal muscle weakness and atrophy of the lower limbs expanding the differential diagnosis to Charcot-Marie-Tooth (CMT) and distal myopathies, especially considering that in the vast majority of patients with dMHN a confirmed genetic diagnosis is not obtained.

In this context, in depth clinical characterization of patients including neurophysiology and muscle magnetic resonance imaging (MRI), can be helpful to confirm the syndromic diagnosis and identify groups of patients with similar features guiding the genetic diagnosis toward already known genes or selecting groups of patients for gene discovery projects[16,17].

We aim to describe the muscle MRI features of 84 dHMN patients with and without molecular diagnosis to identify patterns of fat replacement that could be useful for diagnostic purposes.

2. Methods

2.1. Study setup and subjects

Patients with a clinical and neurophysiological diagnosis of chronic motor neuropathy/neuronopathy from 6 specialized neuromuscular centers were included in the study. The diagnosis of dHMN was based on long slowly progressive history of pure, predominantly distal motor involvement in the lower limbs and neurophysiologic data showing exclusive or almost exclusive involvement of motor nerves associated with a neurogenic pattern in the EMG studies. The study was approved by the Newcastle Hospitals NHS Foundation Trust (Project Number 10833, Caldicott Approval: 7918). A detailed description of the clinical data collected and the genetic studies performed in each patient can be found in the Supplemental Methods section and supplemental Table 1.

2.2. Muscle imaging: acquisition and analysis

All patients were scanned in 1.5 or 3 Tesla MRI machines acquiring T1-weighted (T1w) and T2 weighted Short Tau Inversion Recovery (STIR-T2w) sequences of the lower limbs (LL-MRI) or the whole body (WB-MRI). MRI scans were reviewed by two of the authors with experience on MRI analysis blinded to the clinical data (D.E. and J.D-M). We used the Mercuri score to score that fat present on each muscle [18]. STIR images were scored in all muscles as positive (increased signal within muscles) or negative (absence of signal intensities). A list of muscles scored can be found in the supplemental methods.

2.3. Statistics

We used the Shapiro-Wilk test to assess whether or not our variables were normally distributed. As none of them followed the normal distribution we applied non-parametric statistic tests for the analysis and, therefore, we provide Mercuri score values as median. To investigate correlations between age or disease duration and MRI findings, Spearman's rank correlation was used (coefficient reported as ρ). The correlation was considered significant if p value was less than 0.05 and ρ was 0.6 or higher. Hierarchical analysis and graphical representation as a heatmap were performed using R software (https://www.r-project.org). Statistical analyses were performed using IBM SPSS Statistics, V.21 (IBM, Armonk, New York, USA).

3. Results

3.1. Patients

Eighty-four patients were included in the study. All patients were symptomatic at the time of the MRI except for two patients who were diagnosed based on the presence of *pes cavus* and hyporeflexia with no muscle weakness and electrophysiological studies compatible with pure motor neuropathy. Symptomatic patients had a median onset of symptoms at 30 years (range 1–75) with a median time elapsed since onset of symptoms to the MRI of 14 years (range 1–66).

The most common phenotype of the 82 symptomatic patients was exclusive distal weakness in the lower limbs (LL), present in 37 patients (45.1%), followed by proximal and distal weakness in LL in 16 patients (19.5%) and, proximal and distal weakness in LL plus distal weakness in the upper limbs (UL) in 14 patients (17.1%).

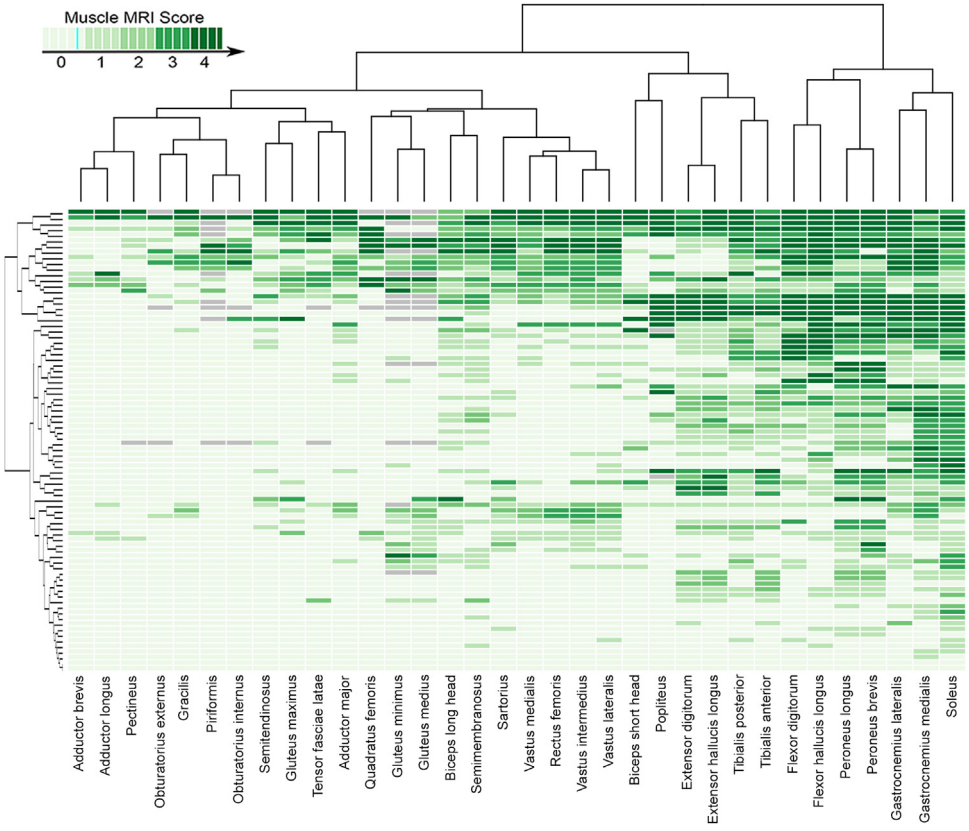

Fig. 1. Heatmap showing muscle fatty replacement of muscles of the pelvis, thigh, and leg.
Patients and muscles are ordered according to unsupervised hierarchical clustering. The score of a muscle in a patient is indicated by the color of the square. The darker the square, the higher the degree of the fat replacement on Mercuri score.

Other combinations were found in 15 patients (Supplemental Table 1). Asymmetric weakness was detected in 24 patients (29.2%).

A causative pathogenic variant was identified in 38 patients (45.2%) while VUS were identified in 8 patients (9.5%) (Supplemental Table 1). The genes in which pathogenic variants were identified were *HSPB1* in 12 patients (14.2%), *DYNC1H1* in six (7.1%), *BICD2* in four (4.7%), *HSPB8* in three (3.5%), *GARS* in two (2.3%), *BSCL2* in two (2.3%), *AARS* in one (1.1%), *WARS1* in one (1.1%), *HARS1* in one (1.1%), *TRPV4* in one (1.1%), *SORD* in one (1.1%), *VRK1* in one (1.1%), *SIGMAR* in one (1.1%), *DGAT2* in one (1.1%) and *KIF5A* in one (1.1%).

3.2. Muscle imaging: general features

We reviewed 27 WB-MRIs containing information of the cranial, upper limbs (UL) and trunk muscles that was normal in 20 patients (74.1%), while only mild changes (Mercuri score 1–2) were observed in the other 7 patients affecting the dorsal and lumbar paraspinal muscles, the abdominal muscles, the subscapularis, the serratus anterior or the latissimus dorsi. One of these patients had a pathogenic variant in the *BICD2* gene, while four patients had VUS in the *SPG11, ARHGEF10, TAF15* or *HARS1* gene respectively. In two patients showing UL and/or trunk involvement genetic studies were negative.

MRI showed fat replacement in almost one LL muscle on T1w imaging in 82/84 patients (97.6%) as shown in the heatmap in Fig. 1. Most of the MRIs showed symmetric involvement, however, asymmetric involvement was found in 20 patients (23.8%). Asymmetric muscles varied and most frequently included the peroneus, soleus and the gastrocnemius (Fig. 2). A distal to proximal gradient of increasing fat replacement was observed in 41 patients (48.8%) being more frequent in the lower leg, alone (23 patients, 30.3%), or in combination with a gradient in the thigh (11 patients, 13.0%). This distal to proximal gradient could be seen in all muscles of the lower legs while in the thigh, it was commonly seen in the vastus lateralis and intermedius (13 patients, 17.1%) and the semimembranosus (9 patients, 11.8%) (Fig. 2).

Muscle texture showed a reticulate pattern on muscles that were mildly replaced by fat that is more evident in muscles that were severely affected towards areas of apparently normal muscle embedded on large confluent areas of fat tissue in more advanced situations This pattern has previously been described as muscle islands[19] (Fig. 2). Muscle islands were identified in 67 patients, being present on the thigh in 36 patients (42.8%), affecting mostly the quadriceps, and on the legs in 57 patients (67.8%) predominantly in the peronei and tibialis anterior.

STIR-T2w imaging was available for 66 patients. Of those, 50 (75.7%) had an increased signal intensity in at least one muscle affecting the lower leg only (36 patients, 54.5%) or in association with the thigh (11 patients, 16.6%) (Fig. 2). The muscles with more frequent STIR abnormalities were the tibialis anterior (38 patients, 57.7%) and the soleus (35 patients, 53.0%). A distal to proximal gradient of STIR-T2w hyperintensity was observed in 27 patients (40.9%) (Fig. 3).

3.3. Pattern description of the lower legs

Fatty replacement of at least one muscle of the lower leg was found in 82/84 patients (97.7%). The superficial posterior compartment was affected in 77 patients (91.6%), followed by the peronei muscles in 70 (83.3%), the deep posterior group in 64 (78.0%) and, the anterior group in 59 (70.2%). The soleus was the

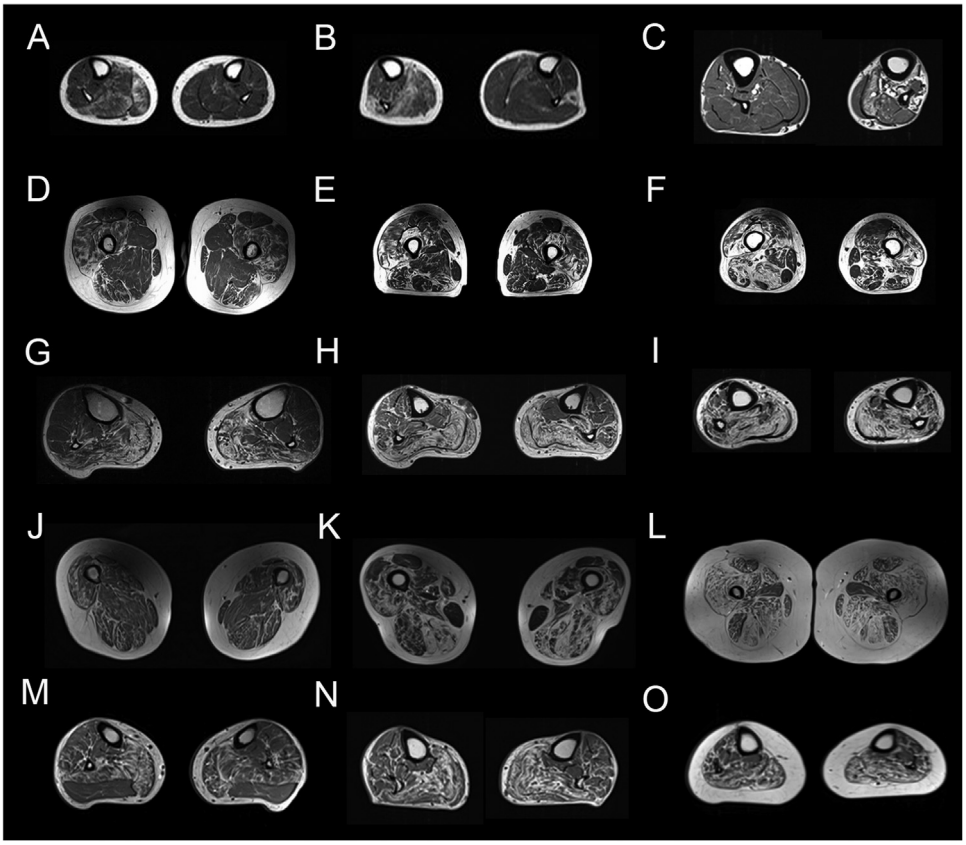

Fig. 2. Common features on T1w imaging of patients with dHMNs. T1w imaging showing characteristic features common to patients with dHMNs. A, B and C, show asymmetric involvement of the muscles of the lower legs. Distal to proximal gradient of fat replacement was observed as show in D to F: D shows an MRI of the thigh at proximal level, E at medium level and, F at distal level. This gradient was also observed in legs, as show in G to I: G shows an MRI of the leg at proximal level, H at medium level and, I at distal level. Examples of the progression of the fat replacement from reticulate pattern in the thigh (J) and leg (M) towards fat islands (K-L and N–O) are also shown.

most frequently affected muscle (75 patients, 89.2%) with a median Mercuri score of 2, although in 50 patients (59.5%), Mercuri score was equal or higher than 2 points. Gastrocnemius medialis (66 patients, 78.5%) and gastrocnemius lateralis (55 patients, 65.4%) followed in frequency of involvement. The least affected muscles were the popliteus (26 patients, 30.9%) and the extensor hallucis longus (48 patients, 57,1%) with an average score 1.2.

Figs. 4 and 5 show a heatmap displaying the lower leg muscles fat replacement scores and helping us to identify a series of patterns:

1 A group of nine patients with severe involvement of all muscles on the legs (Fig. 5B). Of these nine patients, three had pathogenic variants in *HSPB1*, two in the *HSPB8*, one in *TRPV4*, one patient had a VUS in *DCTN1* gene and two patients remain molecularly unresolved.
2 A group of six patients with a relative preservation of the toe extensor muscles despite showing a severe fat replacement of the other compartments (Fig. 5C). Of these six patients, three had pathogenic variants in *BICD2* and three in *DYNC1H1*.
3 A group of 12 patients with a predominant involvement of the superficial posterior compartment of the legs (Fig. 5D). Of these 12 patients, three had pathogenic variants identified: one patient in *GARS*, one patient in *HARS1* and one patient in *VRK1* while nine patients remained undiagnosed.
4 A group of 11 patients with a more severe involvement of the peroneus group in comparison with the rest of the compartments (Fig. 5E). Of these 11 patients, one had a pathogenic variant in *HARS1*, one had a pathogenic variant in *DYNC1H1*, one patient had a VUS in *GDI1* and seven remained without a molecular diagnosis.

3.4. Pattern description of the thigh

Fatty replacement of at least one muscle of the thigh was found in 62 patients (73.8%) being the posterior compartment the most affected in 54 patients (64.2%) followed by the anterior compartment in 47 patients (61.8%). The adductor compartment was the less affected compartment, in 33 patients (39.2%) and, when affected it was to a mild extent (mean Mercuri score of 0.3), being completely preserved in 51 patients (60.7%). The most affected muscle was the vastus lateralis (40 patients, 47.6%), followed by the vastus intermedius (37 patients, 44.0%). The least affected muscles were the adductor brevis (8 patients, 9.5%) and the pectineus (10 patients, 11.9%). Examples of the involvement of the thigh are shown in Fig. 6.

We identified 25 patients who had moderate involvement of the thigh defined as having at least four muscles with fat replacement of 2 or more in the Mercuri score. Of these 25 patients, 16 had a genetic diagnosis: six patients with genetic variants in *DYNC1H1*, three in *HSPB1*, three in *HSPB8*, three in *BICD2*, one in *TRPV4*, one had a VUS in *MFN2* and, seven remained undiagnosed.

3.5. Pattern description of the pelvis

An MRI of the pelvic floor area was available for 80 patients. Fatty replacement of at least one muscle was found in 20 patients

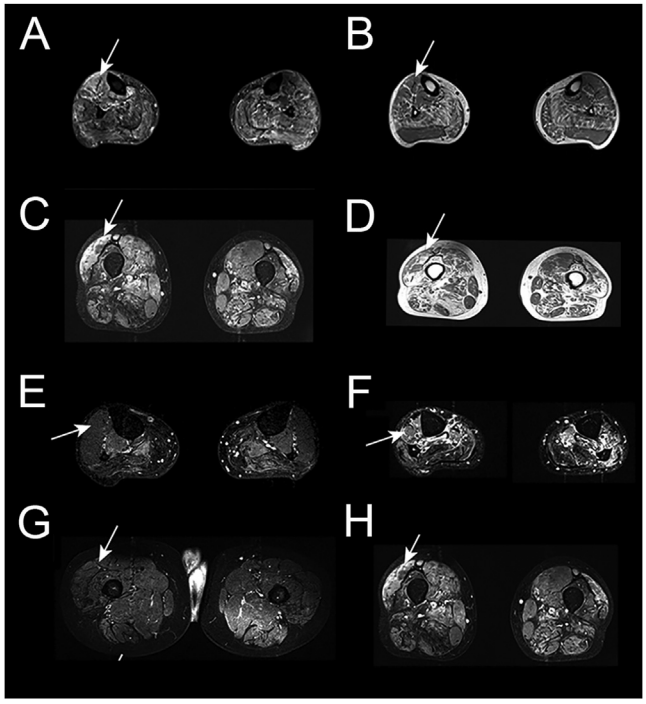

Fig. 3. STIR imaging of lower leg and thigh of patients with dHMNs. Examples of changes observed in the STIR imaging of patients with dHMN. Hyperintensities observed in STIR imaging in the left tibialis anterior of one patient (arrow in A), a muscle nonreplaced by fat in T1w (arrow in E). Hyperintensity observed in STIR imaging in vastus lateralis bilateral and the right adductor magnus on STIR (arrow in B), muscles partially replaced by fat on T1w (arrow in F). A distal to proximal gradient of STIR changes was observed in lower legs (arrows pointing the tibialis anterior in C proximal and G distal) and thigh (arrows pointing the vastus lateralis in D proximal and H distal).

(25.0%). The most frequently affected muscle was the quadratus femoris (12 patients, 15.0%) and the least involved muscle was the obturator externus (9 patients, 11.2%). An MRI covering the entire glutei muscles was available for 69 patients showing fatty replacement of at least one muscle in 29 patients (46.7%) and being the gluteus medius and minimus more often affected that the gluteus maximus.

We identified 22 patients who had moderate involvement of the pelvis defined as having at least four muscles with fat replacement of two or more in the Mercuri score. Fourteen of these 21 patients had a genetic diagnosis: five patients with pathogenic variants in *DYNC1H1* gene, three in *HSPB8* gene, three in *BICD1*, one in *HSPB8* gene, one in *HARS1*, one in *TRPV4*, while one patient had a VUS in *MFN2* and six remained undiagnosed.

3.6. Pattern description by genes affected

We reviewed the pattern of involvement of patients with pathogenic variants in the genes that were more frequent in our cohort. Thirteen patients had variants in *HSPB1* (Fig. 7) and, fat replacement affected non only the distal muscles of the legs but also the proximal muscles and the pelvis in 8 patients. The most affected muscles were the soleus and the peronei group followed by the gastrocnemius medialis and flexor digitorum longus. Eight patients had involvement of the thigh affecting the semimembranosus and biceps femoris long head. Involvement of the vasti muscles was observed only in the more advanced cases. Pelvic muscle involvement was mild, affecting the glutei muscles in three patients. Seven patients had a WB-MRI showing paraspinal, abdominal and serratus anterior involvement in two patients. Upper limb muscles were normal in all HSPB1 patients.

The six patients with pathogenic variants in *DYNC1H1* (Fig. 8) had involvement of the pelvic, thigh and leg muscles. In the pelvis,

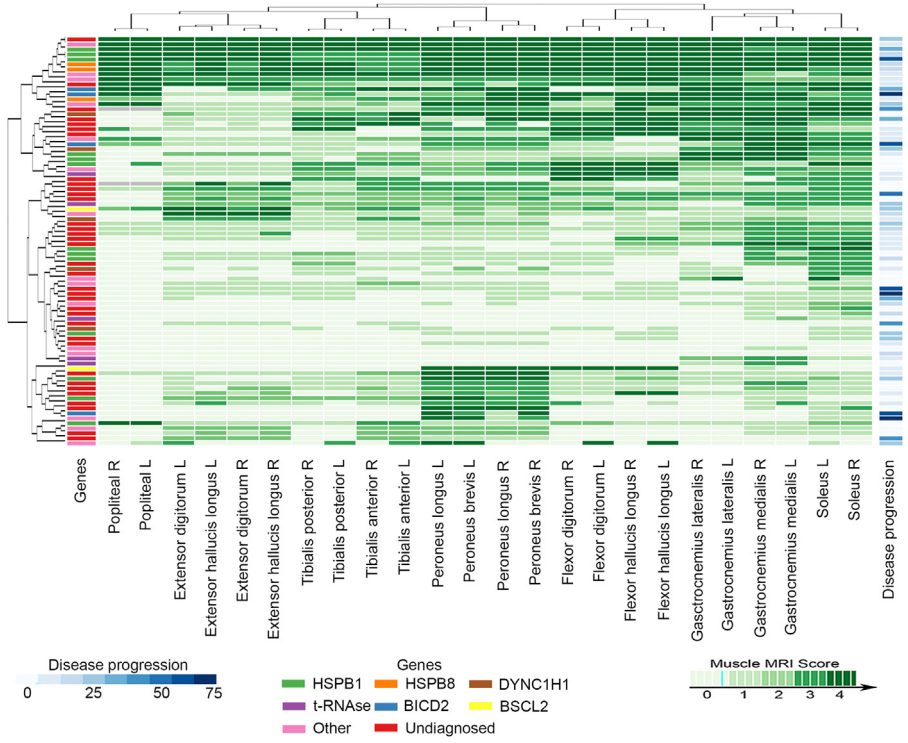

Fig. 4. Heatmap showing muscle fatty replacement of muscles of the legs.
Heatmap showing fat replacement of the muscles of the legs. Patients and muscles are ordered according to hierarchical clustering. The score of a muscle in a patient is indicated by the color of the square. We have included a column with information about the time from onset of symptoms to the MRI (disease progression) in blue on the right. The darker the square, the higher the severity of fat replacement following Mercuri score. We did not find a statistically significant correlation between the median value of the Mercuri score per patient and the years symptomatic. The column on the top left shows the gene that had pathogenic variants.

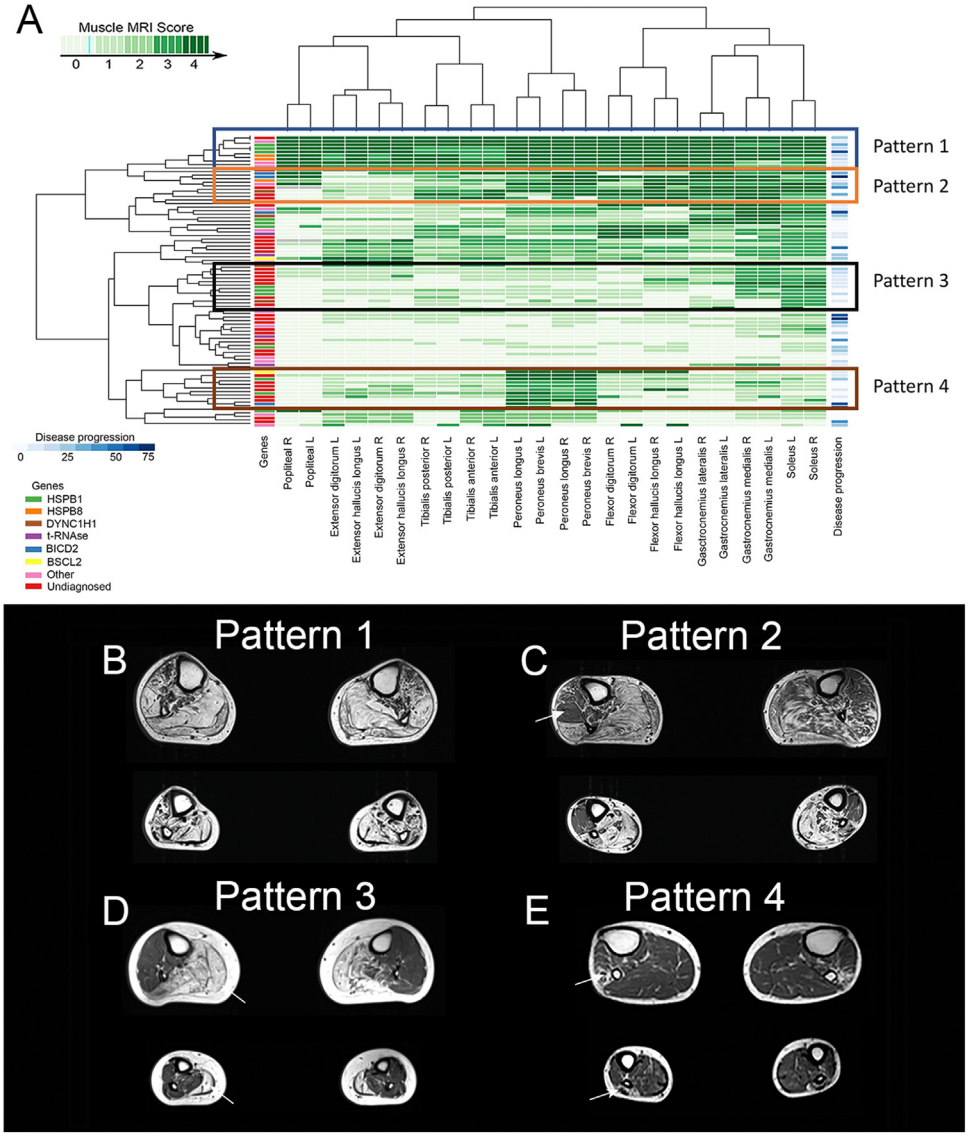

Fig. 5. Patterns of fat replacement of the leg muscles found in the cohort. A) Heatmap showing in different colors four patterns identified in the cohort of patients with dHMN. B) Pattern 1: Global replacement of all muscles, C) Pattern 2: Severe fat replacement of all muscles except toe extensors (arrow). D) Pattern 3: Exclusive involvement of the superficial posterior compartment muscles including soleus and gastrocnemius (arrow). E) Pattern 4: Predominant involvement of the peronei muscle group (arrow).

we observed a predominant involvement of the gluteus minor and medius. In the thigh there was a predominant involvement of the vasti muscles, with sparing of the semitendinosus, biceps short head and adductor longus that was hypertrophic. In the legs we observed a predominant involvement of the gastrocnemius medialis in all patients associated with fat replacement of the peronei muscles in three patients.

We observed fat replacement in the pelvis, thigh and leg muscles in the four patients carrying a pathogenic variant in *BICD2* (Fig. 8). In the lower legs there was a predominant involvement of the soleus, gastrocnemius medialis and lateralis and tibialis anterior with sparing of the toe extensors and peronei (described previously as pattern 2). In the thigh, the vasti muscles, the semimembranosus, biceps femoris long head and the sartorius muscles were involved, while the adductor muscles, semitendinosus and gracilis were spared. The semitendinosus was hypertrophic in all four patients. In the pelvis we observed a predominant involvement of the gluteus minimus and medius muscles. Three cases had a WB-MRI showing mild involvement of the abdominal muscles and the serratus anterior.

We also analyzed as a single group the five patients with pathogenic variants in the aminoacyl-tRNA synthetase genes *GARS1, AARS1, HARS1* and, *WARS1* as these patients showed a common pattern. We observed a predominant involvement of the lower leg muscles, while the thigh and pelvis were affected in just one case an to a mild extent (Fig. 8). In the lower legs, the five patients had a predominant involvement of distal anterior muscles, this is the tibialis anterior and toe extensor (pattern 3), associated to involvement of peronei muscles which in the case of the HARS1 patients was severely replaced by fat (pattern 4). In more advanced patients, we observed the soleus and gastrocnemius to be affected as well. Interestingly, the flexor hallucis longus was spared in all cases. Supplemental figures 1 and 2 show MRIs of single patients with mutations in the least frequent genes of our cohort.

3.7. Correlation between MRI and clinical data

We did not find any correlation between the amount of fat present in the muscles measured using the total MRI score and the age of patients at the time of the MRI ($p = 0.35$, Spearman test) or

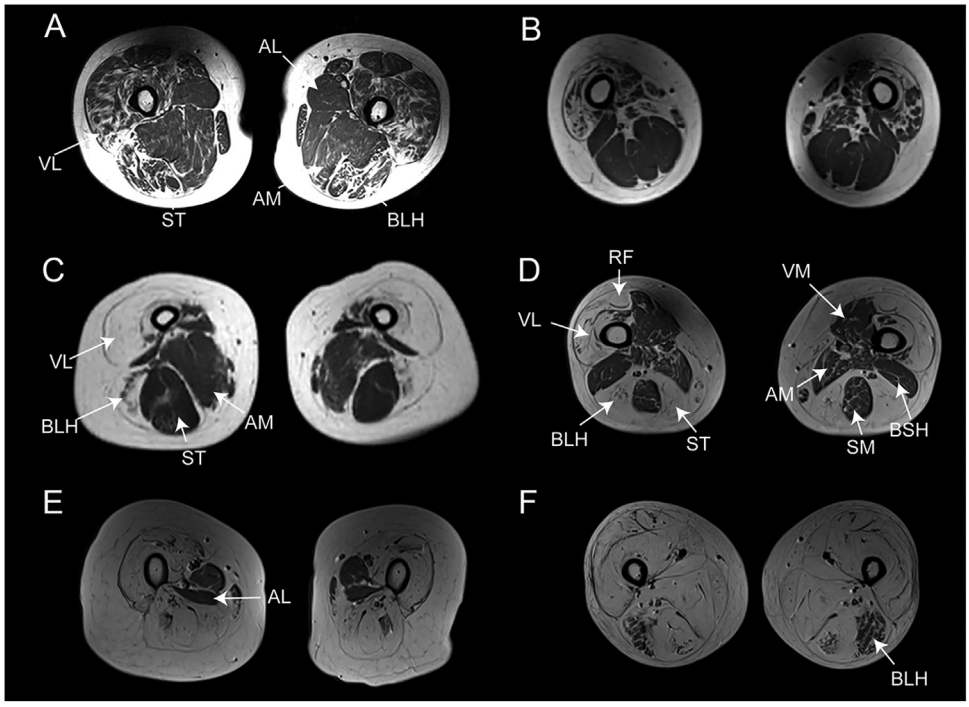

Fig. 6. Examples of involvement of the thigh. A) Fat replacement with reticular pattern affecting the vastus lateralis, semitendinosus and biceps long head but sparing the adductor magnus and longus (arrows). B) Selective involvemenf the anterior compartment of the thigh while posterior muscles are spared. C) Selective involvement of the vasti muscles and biceps long head while adductor longus, magnus and semitendinosus are spared being the later hypertrophic (arrows). D) Predominant involvement of vastus lateralis, rectus femoris, biceps long head and semitendinosus, while vastus medialis, biceps short head and adductor magnus are spared (arrows). E). Severe involvement of all muscles except adductor muscles (arrows). F) Severe involvement of all muscles of the thigh except biceps long head that is moderately affected (arrow).

the time from disease onset ($p = 0.23$, Spearman test). However, we observed significant differences in the MRI score among patients with different degree of motor function involvement, being higher in patients using a wheelchair compared to patients who walked independently, patients requiring splints and patients using a stick or a crutch for walking ($p = 0.004$, Kruskal-Wallis test).

4. Discussion

We have studied a large cohort of patients with dHMN using muscle MRI and identified a series of common features that could help in the diagnosis including the presence of muscle islands, a distal to proximal gradient of fat replacement, frequent asymmetric involvement and increase in the STIR signal. Fat replacement predominated in the muscles of the lower legs and specifically in the posterior compartment while trunk and upper limbs muscles were commonly spared.

The differential diagnosis of dHMNs includes CMT and distal myopathies. Most of CMT patients, opposite to dHMNs, start with distal anterior leg weakness, develop sensory symptoms, and have absent or very reduced amplitude sensory action potentials (SNAPs) facilitating nosology and differential diagnosis with dHMNs[2]. Needle EMG studies show motor unit action potential (MUAPs) of big amplitude and polyphasic with a reduced recruitment pattern [20]. In contrast, patients with distal myopathies can start either with distal anterior or posterior weakness and have no sensory symptoms associated, having normal amplitude SNAPs, but needle examination shows small polyphasic MUAPs and a myogenic recruitment pattern [21]. In patients in advanced stages, distinguishing between neurogenic and myogenic pattern of recruitment on needle EMG could be difficult. Although the combination of clinical examination and EMG studies is helpful, the differential diagnosis between dHMN, CMT and distal myopathies can be complex. In these case complementary tests such as muscle biopsy or muscle MRI can be supportive, especially considering that in most of dHMN patients, a confirmatory genetic diagnosis is not obtained.

There are only a few published studies analyzing the muscle MRI of dHMNs patients and describing findings in patients with mutations in a specific gene [11,22-25]. We have analyzed MRIs of patients with a syndromic diagnosis of dHMN to identify common features that could help in the differential diagnosis with other entities, but also to identify potential characteristic patterns of muscle involvement linked to specific genes.

We have observed some MRI findings to be common to most of the patients analyzed. For example, it is common to identify a reticular pattern in early stages progressing towards what has been called “muscle islands” or “pop-corn appearance” which are areas of muscle spared embedded in large areas of fatty replacement[19]. This finding is characteristic of neurogenic disorders and can also be seen in CMT disease but is infrequent in distal myopathies where fat replacement tends to be homogeneous, except for patients with mutations in the *VCP* gene[26]. A distal to proximal gradient of involvement is also typically seen in neurogenic patients as reflects the dying back phenomenon of the axon, and therefore can be seen as well in CMT patients [27]. An asymmetric degree of fat replacement was observed in 23.8% of dHMN patients, while it is not usually seen in CMT disease [28]. MRI asymmetries can be found in early stages in patients with myofibrillar myopathies, in *ANO5* or *DYSF* patients, facio-scapulo-humeral muscular dystrophy (FSHD) and also in inclusion body myositis (IBM) but are uncommon in other causes of distal myopathies such as mutations in the *MYH7, VCP* or *DNM* genes [29–34]. Hyperintensities on STIR-T2w studies reflects presence of free water in the muscle, which can be seen either in diseases with

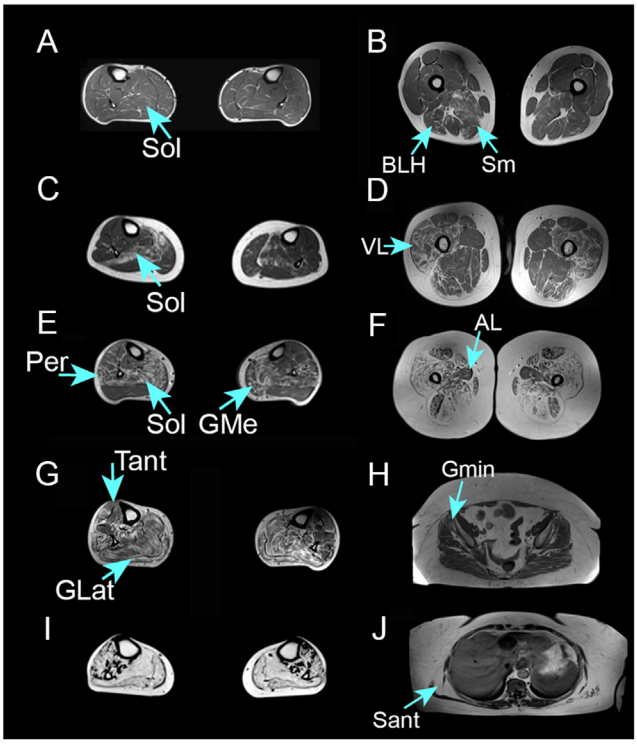

Fig. 7. Muscle MRI involvement in patients with mutations in the HSPB1. Upper row shows examples of progressive muscle fatty replacement in five patients. A) Mild isolated involvement of the soleus (arrow). C) Isolated moderate involvement of the soleus (arrow). E) Moderate fat replacement of soleus, peroneus and gastrocnemius medialis (arrows). G) Expansion of the fat replacement to the tibialis anterior and gastrocnemius lateralis (arrows). I) Severe fat replacement of all muscles of the leg. Bottom row show examples of involvement of thigh, pelvis and trunk area. B) Mild fat replacement of the biceps long head (arrow) and semimembranosus (arrow). D) Expansion of the involvement to the vastus lateralis (arrow). F) Severe fat replacement of all muscles of the thigh, except adductor longus (arrow). H) Pelvic involvement showing mild replacement of gluteus minimus (arrow). J) Trunk involvement showing fat replacement of the serratus anterior (arrow). Sol: soleus, Per: peroneus, GMe: Gastrocnemius Medialis, GLat: Gastrocnemius lateralis, Tant: Tibialis anterior, BLH: Biceps Long Head, Sm: semimembranosus, VL: Vastus Lateralis, AL: Adductor longus, Gmin: Gluteus minimus, Sant: Serratus anterior.

inflammatory infiltrates such as IBM, FSHD, *ANO5* and *DYSF* [35,36]. However, STIR-T2w hyperintensities are also seen in patients with active denervation as is the case of dHMNs [37]. Moreover, we observed a distal to proximal gradient in the hyperintensity on STIR, suggestive of an active length-dependent denervation process which is highly suggestive of a dHMNs/CMT and has not been described in myopathies. Most of the patients with distal myopathies have also involvement of the proximal muscles of the thigh and the trunk, periscapular and upper limb muscles [29,38-40]. In patients with dHMN, upper limb or trunk muscles were normal or only mildly affected. Interestingly, the adductor group on the thigh which tends to be involved from early stages in many myopathies, [31] was spared or only mildly affected in most of the dHMN patients.

The more commonly affected region in dHMN patients was the lower leg muscles. However the muscles affected varied from one patient to the other considerably. We identified 4 patterns of involvement that could be helpful for the diagnostic approach of these patients. For example, pattern 1, in which all leg muscles are severely affected, is common in patients with mutations in *HSPB1* or *HSPB8*. Interestingly, this pattern could be seen in all patients after many years of disease progression, but we have observed that is characteristic of some genes which tend to affect all muscles severely, while other genes spare some regions, such thigh, or muscles even in advanced stages of the disease. Pattern 2, characterized by preservation of the toe extensor muscles despite showing severe fat replacement of all the other leg muscles, is observed in patents with mutations in *BICD2* or *DYNC1H1.* Pattern 3, with predominant involvement of the superficial posterior compartment of the legs, and pattern 4, in which there is more severe involvement of the peroneal group compared to the other compartments, were detected in several patients for which there was not a final genetic diagnosis opening the door to study potential genetic variants shared by these patients. Although fat replacement of the lower leg muscles is predominant and more severe than those of other regions, we observed a clear involvement of pelvic and thigh muscles in some of the patients, especially those with mutations in the *DYNC1H1* and *BICD2* genes, suggesting that these patients can also have involvement outside distal muscles both at the radiological and clinical level as has been previously described. In this sense, the pattern of involvement of patients with mutations in *HSPB1, BICD2* and *DYN1CH1* described here agrees with previous publications confirming the presence of characteristic features that can guide the genetic diagnosis in these cases [11,22,24,41,42].

The reason why some muscles are more affected than others depending on the mutated gene is not known and could depend on many factors. We hypothesized that a common mechanism of action could be responsible of a similar pattern of involvement in patients with variants in different genes. In this sense, variants in the aminoacyl-tRNA synthetase genes *GARS1, AARS1, HARS1* and, *WARS1* might share the same pathogenic pathway and, in agreement with our hypothesis, we observed that patients with variants in these genes shared a common pattern of involvement characterized by predominant involvement of the anterior and peroneal compartment of the leg with no or mild involvement of the thigh and pelvis. In the same way, variants in *DYNC1H1* and *BICD2*, that have been suggested to have a similar mechanism of action, share many commonalities on the MRI [43]. These data open the door to further description of MRI patterns based on the underlying disease's mechanism and not only based on the gene mutated.

Although the wider availability of NGS studies has increased the diagnostic accuracy for neuromuscular diseases, the ratio of dHMNs patients with a confirmed genetic diagnosis is still low compared to for example muscular dystrophies[5,44]. Negative NGS results can be frustrating for both patients and physicians, raising doubts on the syndromic diagnosis of patients and leading to multitude of additional tests that would probably not help on the diagnosis process. Having a muscle MRI compatible with dHMN allows clinicians to confirm a syndromic diagnosis, reducing the anxiety associated with lack of diagnosis and enabling focusing all efforts in identifying the gene responsible of the disease. We have identified unrelated patients with a very similar MRI pattern in whom NGS studies have been negative. We believe that these patients are candidates for joint genetic studies, such as genomes, with the intention of identifying mutations that may be common to them. Furthermore, as we show in this work, if we find pathogenic mutations in novel genes, having an MRI compatible with the diagnosis of dHMN may support the pathogenicity of the mutation.

In conclusion, this study provides a complete understanding of the MRI features observed in patients with dHMN that could be helpful to differentiate them from patients with CMT or distal myopathies accelerating and simplifying the diagnosis process of these patients in daily clinics.

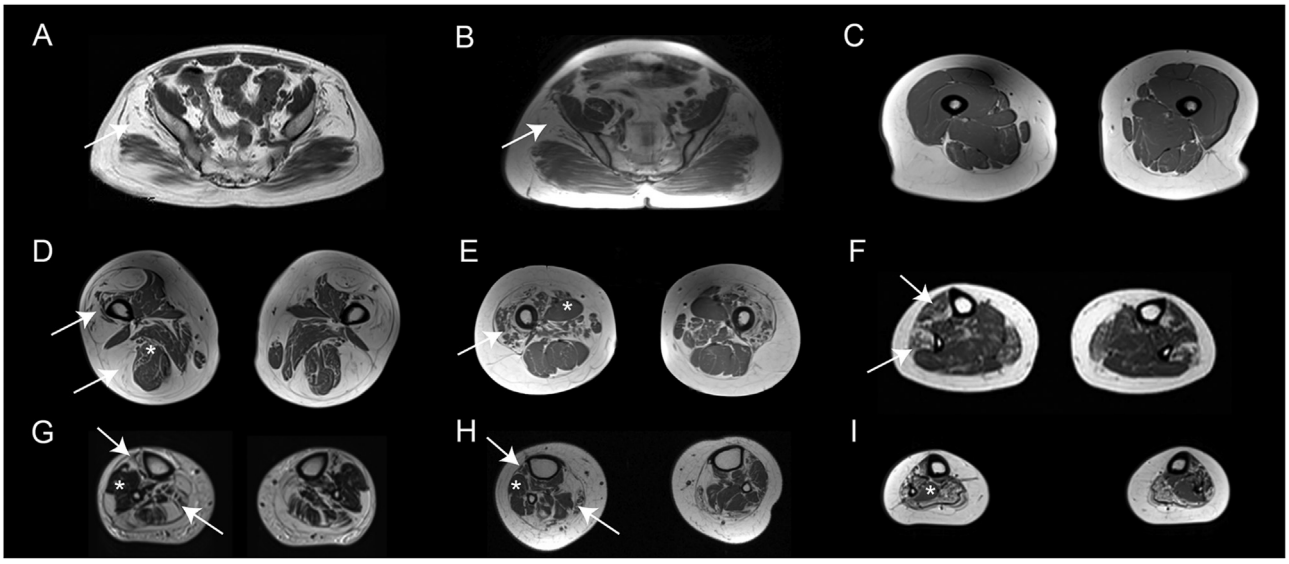

Fig. 8. Muscle MRI involvement in patients with mutations in the *BICD2, DYNC1H1* and aminoacyl-tRNA synthetase genes. A, D and G show involvement of pelvic (A), thigh (D) and leg (G) in patients with mutations in the *BICD2* gene. A) Predominant involvement of the gluteus minimus and medius. D) Involvement of the vasti muscles and the biceps long head (arrows), with sparing of the adductor muscles and the semitendinosus that was hypertrophic (asterisk). G) Involvement of the tibialis anterior, soleus and gastrocnemius muscles (arrows) while the toe extensors are spared (asterisk). B, E and H show involvement of pelvic (B), thigh (E) and leg (H) in patients with mutations in the DYNC1H1 gene. B) Predominant involvement of the gluteus minimus and medius. E) Involvement of the vasti muscles and adductor magnus (arrows), with sparing of the adductor longus that was hypertrophic (asterisk). H) Involvement of the tibialis anterior, soleus and gastrocnemius muscles (arrows) while the toe extensors are spared (asterisk). C, F and I show involvement of the thigh (C) and leg (F and I) in patients with mutations in the aminoacyl-tRNA synthetase genes. C) Thigh muscles were spared in all but one patients. F) Predominant involvement of the tibialis anterior, toe extensor and peroneus muscles in a patient with mutations in the *HARS1* gene (arrows). I) Involvement of all muscles of the distal leg, except flexor hallucis longus that was spared in a patient with mutations in the *GARS1* gene that was spared (asterisk).

Declaration of Competing Interest

Authors have none competing interest

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.nmd.2023.08.010.

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