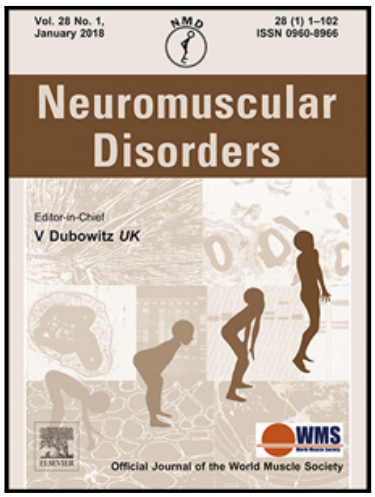


Muscle “islands”: an MRI signature distinguishing neurogenic from myopathic causes of early onset distal weakness

Astrea Guja , M Morrow Jasper , Manzur Adnan , Gunny Roxana , Battini Roberta , Mercuri Eugenio , Reilly Mary M , Muntoni Francesco , Yousry Tarek A

PII: S0960-8966(21)00693-3
DOI: https://doi.org/10.1016/j.nmd.2021.11.003
Reference: NMD 4087

To appear in: *Neuromuscular Disorders*

Received date: 20 July 2021
Revised date: 12 October 2021
Accepted date: 10 November 2021

Please cite this article as: Astrea Guja , M Morrow Jasper , Manzur Adnan , Gunny Roxana , Battini Roberta , Mercuri Eugenio , Reilly Mary M , Muntoni Francesco , Yousry Tarek A , Muscle “islands”: an MRI signature distinguishing neurogenic from myopathic causes of early onset distal weakness, *Neuromuscular Disorders* (2021), doi: https://doi.org/10.1016/j.nmd.2021.11.003

Re[illegible]

Muscle "islands": an MRI signature distinguishing neurogenic from myopathic causes of early onset distal weakness

Astrea Guja*[,a,c], Morrow Jasper M[*,†,b]j.morrow@ucl.ac.uk, Manzur Adnan[c], Gunny Roxana[d], Battini Roberta[a,e], Mercuri Eugenio[f,g], Reilly Mary M[b], Muntoni Francesco[h], Yousry Tarek A[i]

[a]Department of Developmental Neuroscience, IRCCS Stella Maris, Calambrone (Pisa)

[b]Centre for Neuromuscular Diseases, Queen Square UCL Institute of Neurology, Queen Square, London, UK

[c]Dubowitz Neuromuscular Centre, UCL GOS Institute of Child Health

[d]Paediatric neuroradiology, Sidra Medicine, Qatar

[e]Department of Clinical and Experimental Medicine, University of Pisa, Pisa.

[f]Department of Pediatric Neurology, Catholic University, Rome, Italy.

[g]Centro Clinico Nemo, Policlinico Universitario A Gemelli IRCCS, Rome, Italy

[h]NIHR Great Ormond Street Hospital Biomedical Research Centre, Great Ormond Street Institute of Child Health, University College London, & Great Ormond Street Hospital Trust, London, UK.

[i]Neuroradiological Academic Unit, Queen Square UCL Institute of Neurology & Lysholm Department of Neuroradiology, The National Hospital for Neurology and Neurosurgery, UCLH London, UK

[†]**Corresponding author**: Dr Jasper M Morrow, Queen Square Centre for Neuromuscular Diseases, National Hospital for Neurology and Neurosurgery, Queen Square, London WC1N 33BG, United Kingdom

*These authors equally contributed

Abstract

Muscle MRI has an increasing role in diagnosis of inherited neuromuscular diseases, but no features are known which reliably differentiate myopathic and neurogenic conditions. Using patients presenting with early onset distal weakness, we aimed to identify an MRI signature to distinguish myopathic and neurogenic conditions. We identified lower limb MRI scans from

distal myopathy or distal spinal muscular atrophy. An initial exploratory phase reviewed 11 scans from genetically confirmed patients identifying a single potential discriminatory marker concerning the pattern of fat replacement within muscle, coined “islands”. This pattern comprised small areas of muscle tissue with normal signal intensity completely surrounded by areas with similar intensity to subcutaneous fat. In the subsequent validation phase, islands correctly classified scans from all 12 remaining genetically confirmed patients, and 12/13 clinically classified patients. In the genetically confirmed patients MRI classification of neurogenic/myopathic aetiology had 100% accuracy (24/24) compared with 65% accuracy (15/23) for EMG, and 79% accuracy (15/19) for muscle biopsy. Future studies are needed in other clinical contexts, however the presence of islands appears to highly suggestive of a neurogenic aetiology in patients presenting with early onset distal motor weakness.

Keywords

MRI, distal myopathy, distal spinal muscular atrophy, early-onset

Introduction

In patients presenting with muscle weakness, initial localisation of pathology to the peripheral nervous system and subsequent classification as neurogenic or myopathic is generally possible on clinical grounds supported by neurophysiology and laboratory investigations such as creatine kinase [1]. In cases where these data are conflicting or uninformative, muscle biopsy may be helpful but is an invasive investigation and is prone to sampling bias.

In patients with a pure motor distal presentation the distinction between neurogenic and myopathic aetiology can be particularly challenging .The distal myopathies are inherited disorders of muscle with initial or predominant distal limb weakness with mutations in at least *20* different genes identified to [2]. Distal spinal muscular atrophies (SMA), also called distal hereditary motor neuropathies, cover a spectrum of clinically and genetically heterogeneous neurogenic diseases

involve initially and/or predominantly distal limb muscles suggesting a length-dependent disease mechanism. There are at least 26 causative genes of distal motor neuropathies identified to date [3].

In the past decade there has been increasing evidence that muscle MRI may play an important role in the diagnosis of inherited neuromuscular diseases. Specific patterns of muscle involvement have been identified in series of patients with primary muscle diseases including congenital myopathies and muscular dystrophies [4]. Attention has also recently focused on a possible role of muscle MRI in describing patterns of muscle involvement in hereditary neuropathies [5,6]. To date publications of muscle MRI in distal SMA [7–9] and distal myopathies [2,10] have been limited to small case series with no studies to date systematically analysing a series including cases with genetically proven involvement of genes responsible for neurogenic or myopathic conditions. In fact, whether differences on muscle MRI can distinguish myopathic from neurogenic muscle abnormalities has not been systematically assessed in any clinical context.

The aim of this study is to address the diagnostic challenge of distinguishing myopathic from neurogenic diseases in patients with early onset distal weakness. We hypothesised that characteristic MR features could play a central role in accurate diagnostic classification. In the first stage, we assessed multiple imaging features in an exploratory set of patients with genetic confirmation of the myopathic or neurogenic aetiology. In the second stage, we assessed the diagnostic accuracy of a criterion identified in a second larger group of patients with distal onset weakness whilst blinded to genetic or clinical classification as myopathic or neurogenic, to determine the diagnostic accuracy in this separate validation series.

2. Patients and Methods

We [illegible] al (London, UK), Hammersmith Hospital (London, UK) and the IRCCS Stella Maris (Pisa, Italy) obtained from paediatric patients or their affected parents with childhood onset and either the same genetic mutation or clinical phenotype. Scans were included from patients who met the following criteria: i) presentation with predominantly distal lower limb weakness; and ii) pathogenic mutation in known gene or sufficient clinical data to presumptively define aetiology as either neurogenic or myopathic. Exclusion criteria were predominant proximal weakness; or electromyography compatible with myasthenia or myotonia. All MRI scans were performed according to the protocol previously used by our groups using non-contrast axial T1 weighted spin echo images of the lower limbs [11]. All but two patients had both thigh and calf level scans available for assessment. One genetically confirmed neurogenic patient had only thigh imaging, one genetically confirmed myopathic patient had only calf imaging.

MRI scans from a total of 37 patients aged 3-42 years were identified with these inclusion criteria. Nine patients had a genetically confirmed neurogenic condition and 15 patients had a genetically confirmed myopathic disorder. The patients in which a causative gene had yet to be identified were assigned to the “clinically neurogenic” category if there was evidence of neurogenic abnormalities on electromyography and/or muscle biopsy and to the “clinically myopathic” category if there were myopathic abnormalities on electromyography and/or muscle biopsy. By this means 9 patients were classified as clinically neurogenic and 4 patients as clinically myopathic.

2.1. Image Analysis

The study was divided into a first exploratory stage to identify potential MRI features which distinguished neurogenic and myopathic cases, and a second validation stage where any features identified were applied to a separate set of scans with observers blinded to clinical details including diagnosis. During both phases, scans were reviewed by two neurologists (J.M., A.M.)

and [illegible] calf levels, with assessments performed by group consensus.

In the exploratory first stage 11 scans from genetically confirmed cases with both thigh and calf images available (Table 1) were reviewed to determine any discriminatory features with assessors aware of myopathic or neurogenic classification. Initially scans were reviewed for overall quality and presence of artefact precluding accurate assessment. Then they were systematically analysed for features hypothesised to potentially distinguish myopathic and neurogenic aetiology:

- Presence of selective muscle atrophy or hypertrophy
- Presence of a gradient along the length of a muscle
- Notable texture of intramuscular fat accumulation within a muscle [12]
- Any non-muscle imaging features potentially useful diagnostically such as sciatic nerve size [13], and subcutaneous fat thickness

The degree of intramuscular fat accumulation of 24 thigh muscles (left and right rectus femoris, vastus lateralis, vastus medialis, vastus intermedius, long and short heads of biceps femoris, semitendinosus, semimembranosus, adductor longus, adductor magnus, sartorius and gracilis) and 14 calf muscles (left and right tibialis anterior, extensor halluces/digitorum longus, peroneus longus, soleus, medial and lateral heads of gastrocnemius, tibialis posterior) was also assessed using the Mercuri score [14]. However, given the known heterogeneity of muscle involvement even for patients with different mutations in the same gene, detailed assessment of muscle pattern from these scores was not performed. Instead, the Mercuri scores were analysed for features potentially able to distinguish neurogenic from myopathic aetiology were made, specifically:

- Relative involvement of thigh and calf
- Relative involvement of quadriceps and hamstrings
- Relative involvement anterior and posterior lower leg compartments
- Total range of Mercuri grades

In assessment of relative involvement the median Mercuri grade was determined for each muscle group (e.g. thigh and calf) with a difference of at least one grade being considered significant. Consensus data from each subject were collected in a score sheet and subsequently reviewed to identify markers which could reliably distinguish scans from patients with genetically confirmed myopathic and neurogenic aetiology.

The exploratory first phase identified a single potential discriminatory marker between the neurogenic and myopathic patient scans. In the second stage, the same group of examiners made a consensus assessment solely for this discriminatory feature in an additional 26 scans (table 3) blinded to clinical details including their disease classification. This validation set comprised 5 genetically confirmed neurogenic, 8 genetically confirmed myopathic, 9 clinically neurogenic, 4 clinically myopathic patient scans.

Finally, considering the entire cohort of patients with a genetically confirmed diagnosis, diagnostic accuracy was determined for EMG, biopsy and MRI determined classification according to the formula: accuracy = total cases correctly classified / total number of cases.

3. Results

All scans were of sufficient quality to be assessed and overall scan quality was good, with no scans unable to be assessed. Consensus assessments were reached in all patients.

3.1. First stage: analysis of exploratory set

Eleven patient scans were analysed (median age 15y, range 6-42y) comprising four patients with genetically confirmed neurogenic and seven patients with genetically confirmed myopathic

aet[illegible] versus neurogenic aetiology were not found to be helpful with overlap in the findings for the neurogenic and myopathic patients (Table 2).

A previously not described distinctive pattern of intramuscular fat accumulation was observed in all patients with neurogenic aetiology but not in any patients with myopathic aetiology. This pattern comprised small areas of muscle tissue with normal signal intensity completely surrounded by areas where the intensity was similar to subcutaneous fat (Figure 1). We coined the term “islands” to describe the muscle islands within the larger sea of fatty tissue. This pattern was seen only in muscles Mercuri grade 3 or 4. In the neurogenic group, this pattern was present in a variable number of muscles (range 2-24, Table 1) with higher frequency in patients with higher median Mercuri grades. The islands pattern of intramuscular fat accumulation was not seen in any muscles from patients in the myopathic group (Figure 2) and although the overall median Mercuri grade was generally lower, 5 of 7 patients in the myopathic group had muscles of at least Mercuri grade 3.

As the presence of "islands" was the only feature which fully discriminated between the myopathic and neurogenic groups in the exploratory first stage, this was assessed in the validation set of 26 scans. The clinical details of the validation set are outlined in Table 3. In the validation set, the absence or presence of islands correctly classified all genetically confirmed patients as myopathic (8 patients) or neurogenic (4 cases) respectively, matching the 100% accuracy in the exploratory set. Considering the clinically defined cases, the presence or absence of islands was consistent with the clinical classification in 8 of 9 clinically neurogenic cases and all 4 clinically myopathic cases.

Considering the patients with a genetically confirmed diagnosis from both the first and second stage, the diagnostic accuracy of EMG, biopsy and MRI to genetically confirmed neurogenic or myopathic aetiology is detailed in Table 4. MRI by this assessment shows perfect concordance with genetic diagnosis, whereas misclassification is present with both EMG and biopsy.

4. Discussion

In this study we found the appearance of "islands" within moderately to severely fat-replaced muscle to be accurate at distinguishing neurogenic and myopathic genetic causes of early onset distal leg weakness. This texture of fat replacement was identified in an exploratory set of patients and confirmed in an independent validation cohort in which the assessment was performed in a blinded fashion. The presence or absence of "islands" on MRI correctly categorised all genetically confirmed patients, whereas both EMG and muscle biopsy assessment resulted in classifications discordant with the confirmed genetic result in 35% and 21% of cases respectively.

The presence of islands has not previously been reported as an MRI feature which distinguished neurogenic and myopathic conditions; however its presence is evident in publications of early onset distal neurogenic conditions. Small foci of normal muscle within fatty muscle tissue, what we have coined "islands", were noted in 6 patients with *BICD2* mutations who had MRI performed

fro[illegible]2

patients in a single German family [16]. In a series of 9 muscle MRI scans from 30 patients with SMA-LED and *DYNH1C1* mutations islands were evident in the figures, but were not specifically noted by the authors [17]. For *TRPV4*, published MRI scans have shown islands including 2 children [18] and in one patient who underwent MRI from a 4 generation *TRPV4* family [19]. In the latter report, the authors commented on a "feathering" pattern on coronal images of medial gastrocnemius, which may be the appearance of "islands" in longitudinal section. Interestingly, a single patient with a *BICD2* mutation reported to be causing a pure myopathy, was not associated with islands on MRI, suggesting it is specific to the neurogenic pathophysiology [20]. Islands can also be seen in the images from published reports in *SMN1* related SMA [9,21] and distal hereditary motor neuropathy [9]. Hence, although systematic evaluation in a larger cohort is needed to determine the necessary and sufficient conditions for their occurrence, islands seem a consistent feature of early onset motor-neuronopathy whether proximal or distal.

It should be noted that patients presenting with hereditary motor and sensory neuropathies (Charcot-Marie-Tooth disease) were not included in the present study, as reduced sensory responses on nerve conduction studies easily discriminate this condition from distal myopathies. However, islands can be seen in published muscle MRI scans of patients Charcot-Marie-Tooth Disease 1A and 2A [6] and after childhood polio [28]. Thus islands appear to be a potential feature in patients with chronic neurogenic muscle damage due to a range of inherited and acquired causes with childhood onset.

One hypothesis for the pathogenic mechanism resulting in the development of "islands" is that they represent the giant motor units, noted on EMG in these conditions [19]. In conditions where there is significant loss of motor axons, the remaining axons increase both the number and size of the myocytes they innervate by cross sprouting and compensatory hypertrophy, whereas myocytes without innervation degenerate completely and are replaced by fat, resulting in the

obs[illegible] or unit number and size in human skeletal muscle, including tibialis anterior and medial gastrocnemius in the lower limbs. The latter was reported as having 579 motor axons each supplying an average of 1600-1900 myocytes. This resulted in an estimated cross sectional area of a motor unit of 3.4mm^2 in healthy individuals [22]. In patients with SMA, the motor unit number is greatly reduced, to 1-5% of the control value in SMA2, whilst the motor unit size can be up to 15x the control value [23]. The “islands” seen in this study, are of the general size and number expected extrapolating from these data. This hypothesis could be tested by performing neurophysiological motor-unit number estimation and MRI in the same patients.

We did not identify any patients with early onset distal myopathy exhibiting islands. One explanation for this is that islands are only present in muscles at least Mercuri grade 3 and the degree of fat replacement was lower in the myopathic patients in this cohort. However 5/7 of the genetically confirmed patients in the exploratory set had some muscles Mercuri grade 3 and reviewing published images in myopathic conditions, we were similarly unable to identify any islands in patients with myopathic conditions even with severely fat infiltrated muscles. It is important however to distinguish blood vessels which can appear similar to islands in end-stage muscle tissue. In myopathic conditions the T1 weighted MRI muscle changes appear more diffusely distributed, so the pattern is more “moth eaten”, as described in the original Mercuri scale [14]. Other patterns of muscle involvement have been described in genetic muscle diseases and found to be diagnostically helpful, for example the peripheral pattern of fat replacement seen in Bethlem myopathy [12], the semilunar fat replacement in quadriceps in myotonic dystrophy type 1 [24], or a reticular texture with peripheral sparing in some muscles in LGMD2I [25]. However the vast majority of reports on muscle MRI in inherited myopathy, focus on the selective pattern of fat infiltration between muscles, rather than the texture within muscles, so the utility of muscle texture in distinguishing inherited myopathies is largely unknown.

In [redacted] texture analysis of images is an area of increasing research, including muscle MRI [26]. Whilst one goal of quantitative texture analysis is to improve quantitative MRI outcome measure responsiveness, it has been recently applied to successfully distinguish MRI scans between children with Duchenne muscular dystrophy and congenital muscular dystrophy using artificial intelligence (convolutional neural networks) [27] further confirming texture analysis provides diagnostic information.

There were other potentially discriminating factors including relative muscle hypertrophy seen in neurogenic conditions (adductor longus in 3/4 cases), but not myopathic disorders (Table 2); it is also possible that this finding relates to the specific gene defects considered in the exploratory setting therefore cannot be generalized; however in the literature relative hypertrophy of unaffected muscles has also been reported in spinal muscular atrophy due to *SMN1* deletion [4]. However other factors including presence of a gradient of fat replacement within the muscle, or relative involvement of different compartments were not discriminatory. Hypertrophy of muscles can also be difficult to assess as there are no normative data, especially in children.

The major limitation of the present study is the relatively small numbers of genetically confirmed cases, and the result needs to be confirmed in larger studies, and also to define the necessary and sufficient conditions for islands to occur in neurogenic conditions, for example whether islands develop with adult onset neuropathies. However the discriminatory value of islands was strong in both the genetically confirmed and clinically defined cohorts with the only non-concordant case in this study was one clinically neurogenic patient in the validation arm of the study where islands were not observed (patient 17). Although this patient had a clinical diagnosis of spinal muscular atrophy with predominant lower limb involvement, and EMG suggestive of diffuse motor neuropathy, there were other features including pyramidal and cognitive involvement. Further the

sca[illegible] or the development of the islands pattern.

4.1. Conclusions

In conclusion the appearance of “islands” on muscle MRI is a powerful predictor of neurogenic involvement in patients with early onset distal weakness. Further studies are needed to confirm this in broader clinical contexts and to consider the additional diagnostic information which might be obtained from other observed textures of fat replacement within muscles in inherited neuromuscular disorders.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

JMM, MMR, TAY are supported by the National Institute for Health Research University College London Hospitals Biomedical Research. FM is supported by the National Institute for Health Research Great Ormond Street Hospital Biomedical Research Centre

References

[1] Pasnoor M, Dimachkie MM. Approach to Muscle and Neuromuscular Junction Disorders. Contin Lifelong Learn Neurol 2019;25. https://doi.org/10.1212/CON.0000000000000799.

[2] Udd B. Distal myopathies - New genetic entities expand diagnostic challenge. Neuromuscul Disord 2012;22. https://doi.org/10.1016/j.nmd.2011.10.003.

[3] Beijer D, Baets J. The expanding genetic landscape of hereditary motor neuropathies. Brain 2020;143. https://doi.org/10.1093/brain/awaa311.

[4] Mercuri E, Pichiecchio A, Allsop J, Messina S, Pane M, Muntoni F. Muscle MRI in inherited

https://doi.org/10.1002/jmri.20804.

[5] Gallardo E, García A, Combarros O, Berciano J. Charcot-Marie-Tooth disease type 1A duplication: Spectrum of clinical and magnetic resonance imaging features in leg and foot muscles. Brain 2006;129. https://doi.org/10.1093/brain/awh693.

[6] Chung KW, Suh BC, Shy ME, Cho SY, Yoo JH, Park SW, et al. Different clinical and magnetic resonance imaging features between Charcot-Marie-Tooth disease type 1A and 2A. Neuromuscul Disord 2008;18. https://doi.org/10.1016/j.nmd.2008.05.012.

[7] Mercuri E, Messina S, Kinali M, Cini C, Longman C, Battini R, et al. Congenital form of spinal muscular atrophy predominantly affecting the lower limbs: A clinical and muscle MRI study. Neuromuscul Disord 2004;14. https://doi.org/10.1016/j.nmd.2003.09.005.

[8] Berciano J, Baets J, Gallardo E, Zimoń M, García A, López-Laso E, et al. Reduced penetrance in hereditary motor neuropathy caused by TRPV4 Arg269Cys mutation. J Neurol 2011;258. https://doi.org/10.1007/s00415-011-5947-7.

[9] Rossor AM, Morrow JM, Polke JM, Murphy SM, Houlden H, Laura M, et al. Pilot phenotype and natural history study of hereditary neuropathies caused by mutations in the HSPB1 gene. Neuromuscul Disord 2017;27. https://doi.org/10.1016/j.nmd.2016.10.001.

[10] Bugiardini E, Morrow JM, Shah S, Wood CL, Lynch DS, Pitmann AM, et al. The diagnostic value of MRI pattern recognition in distal myopathies. Front Neurol 2018;9. https://doi.org/10.3389/fneur.2018.00456.

[11] Mercuri E, Pichiecchio A, Counsell S, Allsop J, Cini C, Jungbluth H, et al. A short protocol for muscle MRI in children with muscular dystrophies. Eur J Paediatr Neurol 2002;6. https://doi.org/10.1016/S1090-3798(02)90617-3.

[12] Mercuri E, Clements E, Offiah A, Pichiecchio A, Vasco G, Bianco F, et al. Muscle magnetic resonance imaging involvement in muscular dystrophies with rigidity of the spine. Ann Neurol 2010;67. https://doi.org/10.1002/ana.21846.

[13] Sinclair CDJ, Miranda MA, Cowley P, Morrow JM, Davagnanam I, Mehta H, et al. MRI shows

Neurol Neurosurg Psychiatry 2011;82. https://doi.org/10.1136/jnnp.2010.211334.

[14] Mercuri E, Talim B, Moghadaszadeh B, Petit N, Brockington M, Counsell S, et al. Clinical and imaging findings in six cases of congenital muscular dystrophy with rigid spine syndrome linked to chromosome 1p (RSMD1). Neuromuscul Disord 2002;12. https://doi.org/10.1016/S0960-8966(02)00023-8.

[15] Rossor AM, Oates EC, Salter HK, Liu Y, Murphy SM, Schule R, et al. Phenotypic and molecular insights into spinal muscular atrophy due to mutations in BICD2. Brain 2015;138. https://doi.org/10.1093/brain/awu356.

[16] Unger A, Dekomien G, Güttsches A, Dreps T, Kley R, Tegenthoff M, et al. Expanding the phenotype of BICD2 mutations toward skeletal muscle involvement. Neurology 2016;87. https://doi.org/10.1212/WNL.0000000000003360.

[17] Scoto M, Rossor AM, Harms MB, Cirak S, Calissano M, Robb S, et al. Novel mutations expand the clinical spectrum of DYNC1H1-associated spinal muscular atrophy. Neurology 2015;84. https://doi.org/10.1212/WNL.0000000000001269.

[18] Astrea G, Brisca G, Fiorillo C, Valle M, Tosetti M, Bruno C, et al. Muscle MRI in TRPV4-related congenital distal SMA. Neurology 2012;78. https://doi.org/10.1212/WNL.0b013e318245295a.

[19] Oates EC, Reddel S, Rodriguez ML, Gandolfo LC, Bahlo M, Hawke SH, et al. Autosomal dominant congenital spinal muscular atrophy: A true form of spinal muscular atrophy caused by early loss of anterior horn cells. Brain 2012;135. https://doi.org/10.1093/brain/aws108.

[20] Souza PVS, Pinto WBVR, Aivazoglou LU, Cardoso FN, Aihara AY, Yamada AF, et al. Distal myopathy due to BICD2 mutations. Clin Neurol Neurosurg 2018;165. https://doi.org/10.1016/j.clineuro.2017.12.023.

[21] Sproule DM, Montgomery MJ, Punyanitya M, Shen W, Dashnaw S, Montes J, et al. Thigh muscle volume measured by magnetic resonance imaging is stable over a 6-month interval in spinal muscular atrophy. J Child Neurol 2011;26.

[22] FEINSTEIN B, LINDEGARD B, NYMAN E, WOHLFART G. Morphologic studies of motor units in normal human muscles. Acta Anat (Basel) 1955;23. https://doi.org/10.1159/000140989.

[23] Bromberg MB, Swoboda KJ. Motor unit number estimation in infants and children with spinal muscular atrophy. Muscle and Nerve 2002;25. https://doi.org/10.1002/mus.10050.

[24] Kornblum C, Lutterbey G, Bogdanow M, Kesper K, Schild H, Schröder R, et al. Distinct neuromuscular phenotypes in myotonic dystrophy types 1 and 2: A whole body highfield MRI study. J Neurol 2006;253. https://doi.org/10.1007/s00415-006-0111-5.

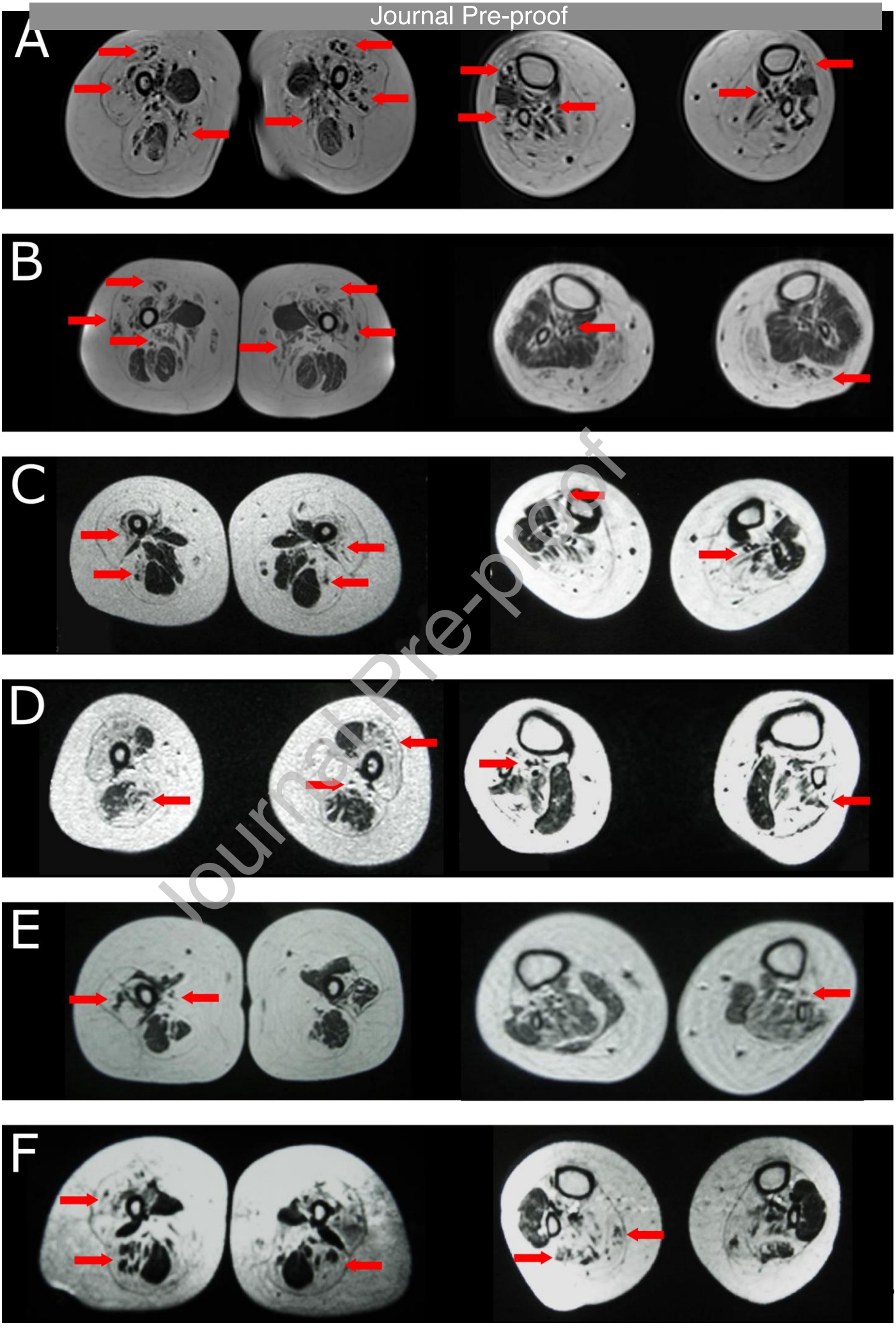
[25] Willis TA, Hollingsworth KG, Coombs A, Sveen ML, Andersen S, Stojkovic T, et al. Quantitative magnetic resonance imaging in limb-girdle muscular dystrophy 2i: A multinational cross-sectional study. PLoS One 2014;9. https://doi.org/10.1371/journal.pone.0090377.

[26] De Certaines JD, Larcher T, Duda D, Azzabou N, Eliat P-A, Escudero LM, et al. Application of texture analysis to muscle MRI: 1-What kind of information should be expected from texture analysis? EPJ Nonlinear Biomed Phys 2015;3. https://doi.org/10.1140/epjnbp/s40366-015-0017-1.

[27] Cai J, Xing F, Batra A, Liu F, Walter GA, Vandenborne K, et al. Texture analysis for muscular dystrophy classification in MRI with improved class activation mapping. Pattern Recognit 2019;86. https://doi.org/10.1016/j.patcog.2018.08.012.

[28] Tollbäck A, Söderlund V, Jakobsson F, Fransson A, Borg K, Borg J. Magnetic resonance imaging of lower extremity muscles and isokinetic strength in foot dorsiflexors in patients with prior polio. Scand J Rehabil Med. 1996 Sep;28(3):115-23.

Figure captions



Fig[illegible]c conditions. Note the muscle islands (red arrows). A: TRPV4, B: BICD2, C: DYNC1H1, D-F: clinically neurogenic

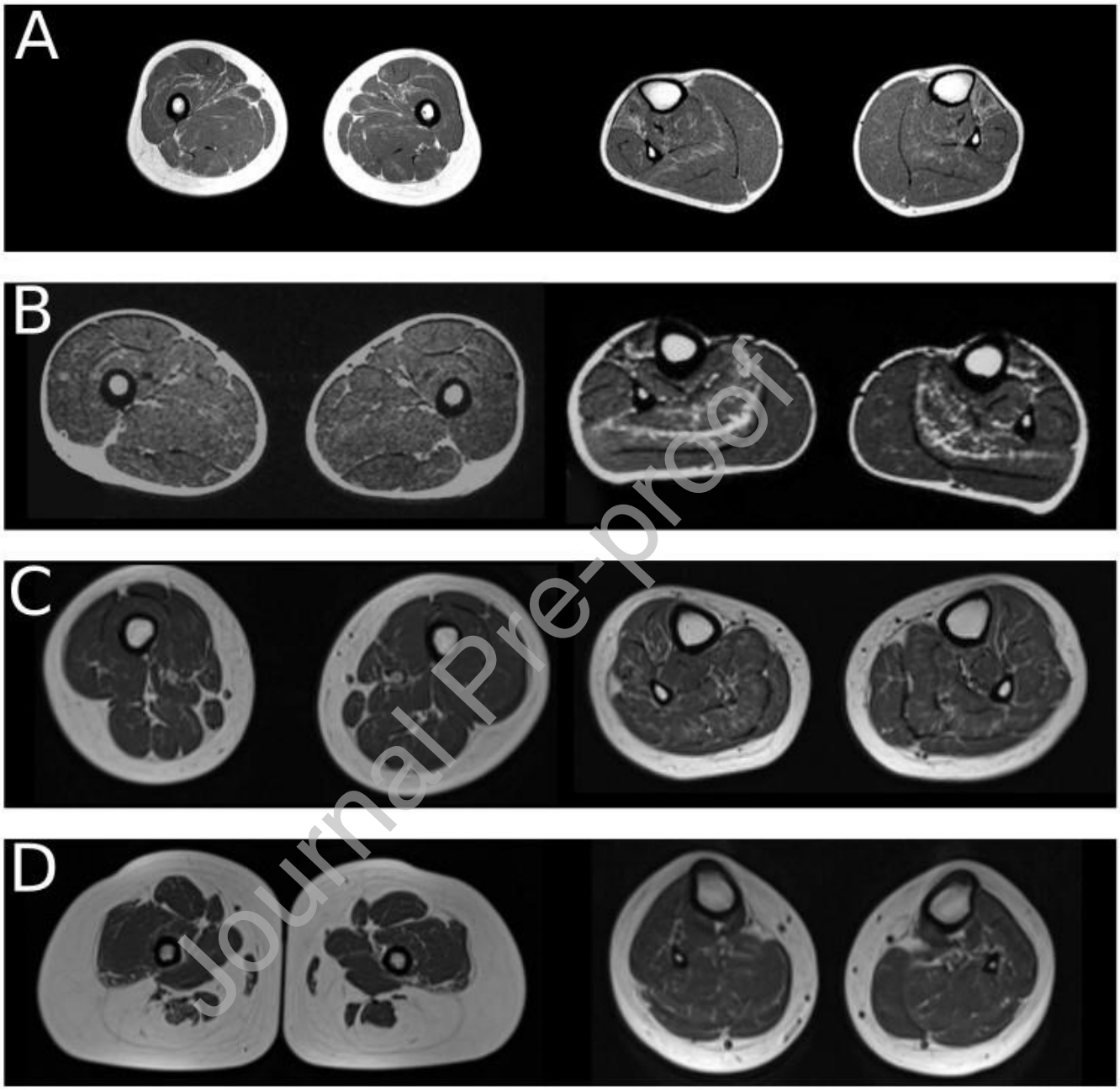


Figure 2. Muscle MRI at thigh (left) and calf (right) level in genetically confirmed myopathic conditions. No muscle islands are seen. A: MYH7, B: DES, C: MYH2, D: NEB

Table captions

Tab

EMG: electromyography; N: neurogenic; M: myopathic; NP: not performed; D: dystrophic

Pt	Age	Gene	EMG	Biopsy	Clinical findings	Median Mercuri grade		Number of Muscles "Islands" present
						Thigh	Calf	
Genetically confirmed neurogenic								
1	38y	DYNC1H1	N	N	Very mild distal weakness	2a	2a	2/38
2	15y	DYNC1H1	N	M	Arthrogryposis, lower limb weakness, lower leg atrophy	4	3/4	15/38
3	10y3m	DYNC1H1	M	M	Lower limb weakness with lower leg atrophy	4	4	24/38
4	11y2m	DYNC1H1	M	NP	Marked distal muscular atrophy and weakness	4	3	14/38
Genetically confirmed myopathic								
5	15y6m	MYH7	M	M	Pes cavus, mild distal weakness	1	1	0/38
6	8y6m	MYH2	M	M	Mild scapulo-peroneal weakness	2a	2a	0/38
7	6y6m	NEB	M	M	Severe distal weaknes, bilateral foot drop	2a	2b	0/38
8	15y	MYH7	M	D	Mild scapulo-peroneal weakness	2a	1/2a	0/38
9	16y9m	MYH7	M	NP	Mild scapulo-peroneal weakness	1	1	0/38
10	42y	MYH7	N	M	Mild scapulo-peroneal weakness	1	2a/2b	0/38
11	16y	MYH7	N	NP	Lower limb weakness with lower leg atrophy	1	2a	0/38

Table 2: Assessment of possible discriminators between neurogenic and myopathic groups

Feature	Myopathic group	Neurogenic group
Selective muscle hypertrophy or atrophy	Selective atrophy 6/7 Not present 1/7	Mixed atrophy/hypertrophy 3/4 Not present 1/4
Presence of a gradient of along the length of a muscle	Distal 2/7 None 5/7	None 4/4
Presence of “Islands” texture within any muscle	Present 0/7 Absent 7/7	Present 4/4 Absent 0/4
Non-muscle features: nerve size, subcutaneous fat thickness	None noted	None noted
Relative involvement of thigh and calf	Thigh > Calf 0/7 Thigh = Calf 4/7 Thigh < Calf 3/7	Thigh > Calf 1/4 Thigh = Calf 3/4 Thigh < Calf 0/4

quadriceps and hamstrings	Quadriceps = Hamstrings 6/7 Quadriceps < Hamstrings 1/7	Quadriceps = Hamstrings 1/4 Quadriceps < Hamstrings 0/4
Relative involvement anterior and posterior lower leg compartments	Anterior > Posterior 6/7 Anterior = Posterior 0/7 Anterior < Posterior 1/7	Anterior > Posterior 0/4 Anterior = Posterior 2/4 Anterior < Posterior 2/4
Total range of Mercuri grades	3 grades: 3/7 4 grades: 2/7 5 grades:2/7	4 grades: 1/4 5 grades: 1/4 6 grades 2/4

Table 3: clinical and MRI data in validation set. All patients were ambulant unless otherwise noted.

EMG: electromyography; N: neurogenic; M: myopathic; NP: not performed

Pts	Age	Gene	EMG	Biopsy	Clinical findings	Islands
Genetically confirmed neurogenic						
12	10y6m	TRPV4	N	N	Arthrogryposis, predominant lower limb weakness, non-ambulant	Yes
13	3y5m	DYNC1H1	N	N	Arthrogryposis	Yes
14	5y8m	BICD2	N	NP	Mild scapulo-peroneal weakness	Yes
15	8y9m	BICD2	N	M	Arthrogryposis, distal lower limb atrophy, non-ambulant	Yes
16	39y3m	BICD2	M	M	Mild scapulo-peroneal weakness	Yes
Clinically Neurogenic						
17	7y5m		N	N	Distal lower limb atrophy and mild TA tightness	No
18	8y3m		N	N	Scapulo- peroneal and hip girdle weakness	Yes
19	10y9m		N	N	Distal weakness, congenital talipes equinovarus	Yes
20	17y2m		N	Normal	Arthrogryposis, lower limb atrophy	Yes
21	41y 2m		N	NP	Distal weakness with foot drop	Yes
22	2y6m		N	NP	Congenital talipes, muscle atrophy	Yes
23	11y3m		N	NP	Mild weakness in the lower limbs	Yes
24	9y8m		ND	N	Arthrogryposis, very severe distal limbs weakness	Yes
25	14y4m		N	N	Arthrogryp.mild weakness in the lower limbs	Yes
Genetically confirmed myopathic						
26	14y	TTN	M	M	Distal weakness with foot drop	No
27	4y6m	NEB	M	M	Distal weakness with foot drop	No
28	23y	NEB	M	M	Distal weakness with foot drop	No
29	19y	BAG3	M	NP	Severe weakness of lower limbs with foot-drop	No
30	12y	TPM3	Normal	M	Mild distal myopathy	No
31	12y2m	BAG3	N	M	Lower limbs weakness with foot-drop	No
32	15y1m	BAG3	N	M	Bilateral pes cavus and tiptoe walking, distal hand weakness	No
33	17y	MYH7	N	M	Distal weakness with foot drop	No
Clinically myopathic						
34	36y5m		M	M	Marked distal weakness	No
35	13y4m		M	M	Scapulo-peroneal weakness	No

36						
37	10y10m		M	M	Mild distal weakness	No

Table 4: Overall accuracy of classification by EMG, biopsy and MRI with reference to genetic classification. Results where EMG or biopsy were not performed or were normal are excluded from analysis.

		EMG classification	
		Myopathic	*Neurogenic*
Genetic classification	*Myopathic*	9	5
	Neurogenic	3	6
Overall accuracy of EMG classification: 65% (15/23)			
		Biopsy classification	
		Myopathic	*Neurogenic*
Genetic classification	*Myopathic*	12	0
	Neurogenic	4	3
Overall accuracy of biopsy classification: 79% (15/19)			
		MRI classification	
		Myopathic	*Neurogenic*
Genetic classification	*Myopathic*	15	0
	Neurogenic	0	9
Overall accuracy of MRI classification: 100% (24/24)			