Journal of Neurology (2019) 266:2524–2534
https://doi.org/10.1007/s00415-019-09437-3

ORIGINAL COMMUNICATION

HNRNPDL-related muscular dystrophy: expanding the clinical, morphological and MRI phenotypes

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Received: 23 March 2019 / Revised: 16 June 2019 / Accepted: 18 June 2019 / Published online: 2 July 2019

Abstract

Autosomal dominant limb girdle muscular dystrophy D3 HNRNPDL-related is a rare dominant myopathy caused by mutations in *HNRNPDL*. Only three unrelated families have been described worldwide, a Brazilian and a Chinese carrying the mutation c.1132G>A p.(Asp378Asn), and one Uruguayan with the mutation c.1132G>C p. (Asp378His), both mutations occurring in the same codon. The present study enlarges the clinical, morphological and muscle MRI spectrum of AD-*HNRNPDL*-related myopathies demonstrating the significant particularities of the disease. We describe two new unrelated Argentinean families, carrying the previously reported c.1132G>C p.(Asp378His) *HNRNPDL* mutation. There was a wide phenotypic spectrum including oligo-symptomatic cases, pure limb girdle muscle involvement or distal lower limb muscle weakness. Scapular winging was the most common finding, observed in all patients. Muscle MRIs of the thigh, at different stages of the disease, showed particular involvement of adductor magnus and vastus besides a constant preservation of the rectus femoris and the adductor longus muscles, defining a novel MRI pattern. Muscle biopsy findings were characterized by the presence of numerous rimmed vacuoles, cytoplasmic bodies, and abundant autophagic material at the histochemistry and ultrastructural levels. *HNRNPDL*-related LGMD D3 results in a wide range of clinical phenotypes from the classic proximal form of LGMD to a more distal phenotype. Thigh MRI suggests a specific pattern. Codon 378 of *HNRNPDL* gene can be considered a mutation hotspot for *HNRNPDL*-related myopathy. Pathologically, the disease can be classified among the autophagic rimmed vacuolar myopathies as with the other multisystem proteinopathies.

Keywords LGMDD3 *HNRNPDL*-related · *HNRNPDL* gene · Rimmed vacuolar myopathy · Autophagy

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Introduction

According to the recent definition established by a group of experts at the 229th European Neuromuscular Centre (ENMC)international workshop in 2017: 'Limb girdle muscular dystrophy (LGMD) is a genetically inherited condition that primarily affects skeletal muscle leading to progressive, predominantly proximal muscle weakness at presentation' [1]. Among LGMD, the autosomal dominant forms are relatively rare and represent 10% of all LGMD [2]. According to the new LGMD classification, autosomal dominant LGMD includes: LGMDD1 DNAJB6-related, LGMDD2 TNPO3-related, LGMDD3 HNRNPDL-related and LGMDD4 CAPN3-related myopathy [1].

LGMDD3 HNRNPDL-related, previously LGMD1G (OMIM #609115) was first described in 2004 in a dominant Brazilian-Caucasian family with 12 patients distributed over three generations [2]. The age at onset varied from 30 to 47 years. The slowly progressive disease started with proximal lower limbs involvement later followed by proximal upper limbs weakness. Progressive finger and toe flexion limitations and muscle cramps were present in most patients. Serum creatinekinase (CK) activity was normal or slightly increased. Muscle histology was available for one patient and described as a "myopathic pattern" with fibre size variability, necrotic fibres, groups of atrophic angulated fibres, and rimmed vacuoles [2]. In 2014, LGMDD3HNRNPDL-related was also reported in a Uruguayan family with 18 affected members [3].The age of onset varied from 15 to 55 years of age. The clinical signs were similar to those of the Brazilian family, but in 8 patients the symptoms started with proximal upper limb weakness and difficulty to raise the arms. Additionally, some affected members presented early onset cataracts [3].For both the Brazilian and Uruguayan families; the disease affected both sexes with incomplete penetrance. Genome sequencing identified two different missense mutations in *HNRNPDL* (on chromosome 4q21) encoding the heterogeneous nuclear ribonucleoprotein D-like (hnRNPDL). Both mutations were heterozygous and affected the same aminoacid: p.(Asp378Asn) in the Brazilian family and p.(Asp378His) in the Uruguayan family. Recently, a Chinese family with 10 affected individuals over three generations was reported to carry the original Brazilian p.(Asp378Asn) mutation [4].While muscle morphology showed similar features with the presence of rimmed vacuoles, the clinical phenotype slightly differed from the previously reported families with half of the Chinese patient's presenting distal as well as proximal limb weakness. Flexion limitation of fingers and toes, which was a typical feature in the other two reported families, was not present, highlighting the broad clinical spectrum of the disease.

hnRNPDL acts as a regulator of pre-mRNA splicing and nuclear export. *HNRNPDL* mutations are thought to cause defects in mRNA biogenesis and metabolism [5].This DNA/RNA-binding protein has been shown to localize in the cytoplasm and in the nucleus [6, 7]. Data from Vieira et al. 2014, suggested that the translocation of the protein from the cytoplasm to the nucleus might be impaired by the p.(Asp378Asn)mutation. This could prevent the protein to access to the nucleus to perform its function on pre-mRNA maturation. Many hnRNPs were linked to various diseases due to their crucial role in the regulation of gene expression [8]. Mutations in the prion-like domains (PrLD) of hnRNPA1 and hnRNPA2B1 cause a multisystem proteinopathy (MSP), an inherited pleiotropic degenerative disorder that can affect muscle, bone, and the nervous system. However, an isolated myopathy has also been described in association with hnRNPA1 mutations [9]. Affected tissues in multisystem proteinopathies present ubiquitin-positive inclusions that contain RNA-binding proteins, such as hnRNPA1 and hnRNPA2B1, and may stain positive for proteins that mediate ubiquitin-dependent autophagy [9]. Rimmed vacuolation is a key feature of the myopathology in MSP [10].

Here we describe two unrelated families from Argentina with LGMDD3 HNRNPDL-related at the clinical, muscle imaging, histopathological and ultrastructural level.

Materials and methods

Patients

Two unrelated Argentinean families of European ancestry with history of adult-onset myopathy were included in the study (Fig. 1: family A and family B). All patients underwent detailed clinical examination summarized in Table1.

Morphological studies

Open muscle biopsies of the quadriceps were obtained from four patients, three (III-1, II-6 and II-9) from family A (FA) and one (III-1) from family B (FB) (Fig. 1). For conventional histochemical techniques 10-µm thick cryostat sections were stained with haematoxylin and eosin (HE), modified Gomori trichrome (mGT), Periodic-acid Schiff technique (PAS), Oil red O, nonspecific esterase, reduced nicotinamide adenine dinucleotide dehydrogenase-tetrazolium-reductase(NADH-TR), succinic dehydrogenase (SDH), cytochrome c oxidase (COX), menadione-nitro blue tetrazolium and adenosine triphosphatase (ATPase) pre-incubated at pH 9.4, 4.63, 4.35.

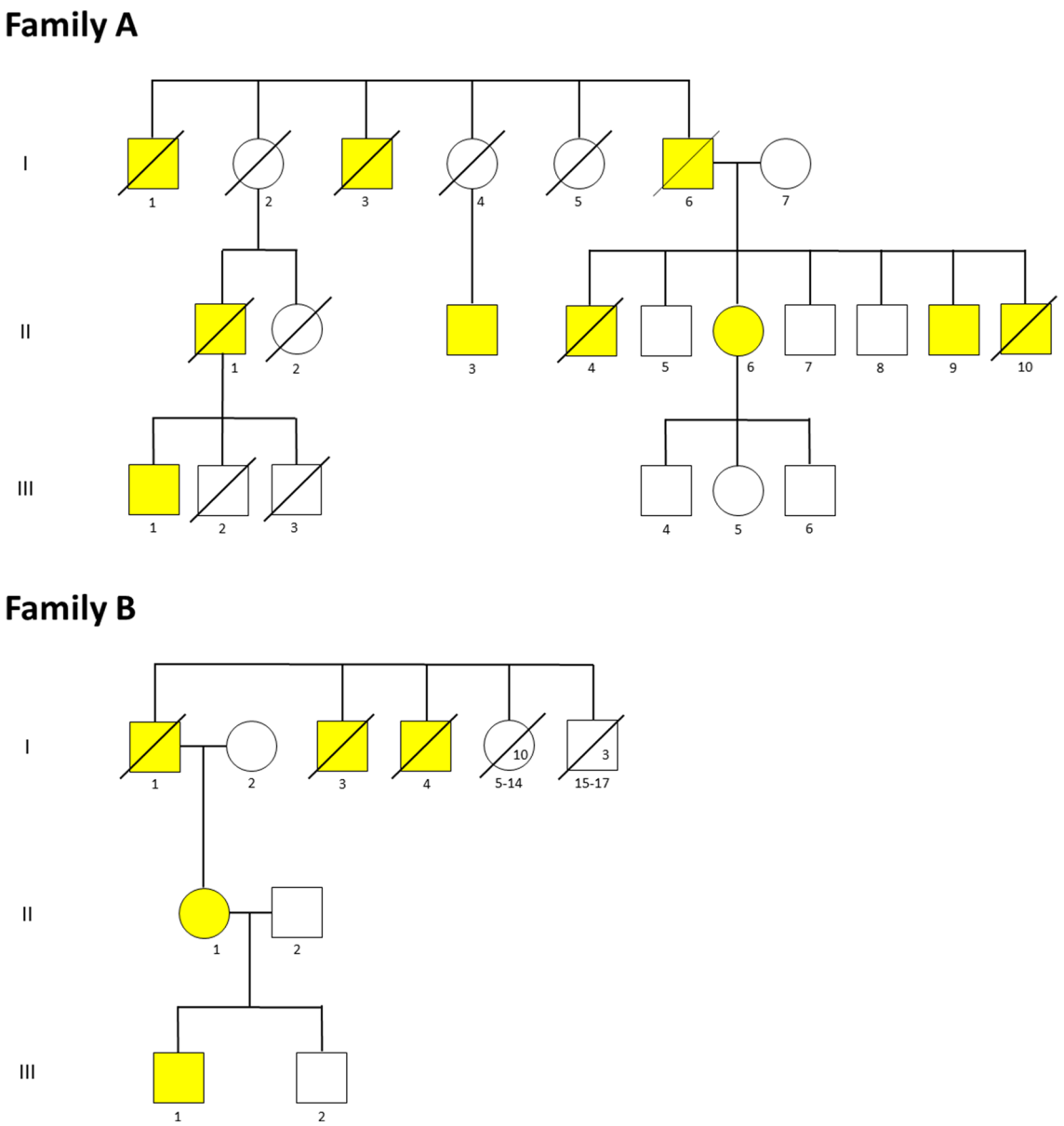

Fig. 1 Family A and Family B pedigrees

Immunohistochemistry (IHC)

IHC was performed in frozen muscle sections from patients III-1, II-6, II-9from FA. Antibodies against Desmin (Anti-Human Desmin, Clone D33, Dako Laboratories, Denmark A/S), Myotilin (NCL-Myotilin, Novocastra Laboratories, Newcastle Upon Tyne, United Kingdom), αB-crystallin (CRYAB, GeneTex International Corporation, Irvine, USA), C5b9 (Dako), P62 (BD-Transduction Laboratories) and antibodies against dystrophin (Dys2, Leica), a, b, g, d sarcoglycans (Leica), alpha 2 laminin, dysferlin (Leica), were applied to 10 µm thick cryosections and revealed using immunoperoxidase techniques.

Electron microscopy

Electron microscopy (EM) was performed in three cases (FA.III-1,FA.II-6 and FB.III-1).Small muscle samples were fixed in 2.5% glutaraldehyde, pH 7.4, post-fixed in 4% osmium tetroxide, dehydrated and embedded in araldite. Several blocks from each patient were studied, including longitudinally and transversally oriented samples. Semi-thin sections were stained with toluidine-blue and examined on light microscopy. Ultrathin sections were stained with uranyl acetate and lead citrate (Reynolds) and viewed with a Zeiss EM 109 T.

Table 1 Clinical, MRI and histological findings in the patients

Patient	FA.III-1	FA.II-6	FA.II-9	FB.II-1	FB.III-1
Age (y)	56	68	59	65	46
Age at onset (y)	38	48	38	63	41
Sex	M	F	M	F	M
Weakness	Early distal LL, then proximal LL and UL	Early proximal LL, then distal UL and LL	Early proximal LL, then distal UL and LL	Slight proximal LL	Proximal LL, proximal UL
Scapular winging	+	+	+	mild	+
Limitation of fingers flexors	+	+	+	−	−
Thigh atrophy	+	+	+	−	+
Early cataracts	−	+	−	−	−
MRI pattern	NP	RF/AL preserved Tibial anterior and soleus involvement	NP	RF/AL preserved No lower leg involvement	RF/AL preserved No lower leg involvement
Histology findings	Numerous rimmed vacuoles, cytoplasmic bodies and atrophic fibres	Rimmed vacuoles. Few necrotic/regenerative fibres. Some atrophic fibres and pyknotic nuclear clumps	Numerous rimmed vacuoles	NP	Scarce rimmed vacuoles Slight subsarcolemmal increase in oxidative activity was present. Isolated atrophic fibres
Electron microscopy findings	Abundant autophagic changes, cytoplasmic bodies, myofibrillar degeneration, filamentous bundles of Z-disc origin	Some autophagic changes with myofibrillar degeneration and filamentous accumulation	NP	NP	Clear autophagic material and filamentous structures
CK levels	42y: 1123–1800 IU/L 54y: 677 U/L	300 IU/L	N.P	Normal	862 IU/L(upper normal limit 150 IU/L)
Echocardiogram	Normal	Normal	Normal	Normal	Normal
Spirometry	Normal	Normal	VI; diminished VC	Normal	Normal

NP not performed, *LL* lower limbs, *UL* upper limbs, *RV* rimmed vacuoles, *Y* year, *CK* creatine kinase, *VC* vital capacity, *VI* ventilatory insufficiency, *RF* rectus femoris muscle, *AL* adductor longus muscle

Genetic analysis

In family A (Fig. 1), exome sequencing was performed for individuals FA.II-6, FA.II-7 and FA.II-9 with the SureSelect Human All Exon 50 Mb capture library v5 (Agilent, Santa Clara, USA). Samples were paired-end sequenced on Illumina HiSeq 2500 (Illumina, San Diego, USA). In family B (Fig. 1), exome sequencing was performed on the index patient (FB.III-1) with Roche SeqCap EZ Human Exome v3 with 75 bp paired end reads and sequenced on Illumina NextSeq 550 (Illumina, San Diego, USA).

Alignment was performed on the GRCh37/hg19 reference genome and the variants were filtered using their frequency in gnomAD (https://gnomad.broadinstitute.org/) and in our in-house database containing over 1500 exomes. The variants with minor allele frequency over 0.01 were removed from analysis. The mutation was numbered according to the GenBank NM_031372.3 with +1 corresponding to the A of the ATG translation initiation codon.

In family A, confirmation of variants and segregation was performed with Sanger sequencing for four affected (FA. II-6, FA.II-9, FA.II-10, FA.III-1) and three healthy individuals (FA.II-5, FA.II-7, FA.III-2). In family B, the segregation was verified by Sanger sequencing in three other family members (FB.II-1, FB.II-2 and FB.III-2).

Magnetic resonance imaging (MRI)

Muscle MRI was performed in one member from family A (FA.II-6) and two from family B (FB.III-1, FB.II-1). Fast Spin Echo (FSE) T1 and T2-weighted STIR (Short-tau inversion recovery) images were acquired in axial and coronal planes of both thighs and legs. We analysed and classified all cases considering the type of involvement of each muscle: perifascial, concentric, mottled, muscle atrophy vs. hypertrophy, oedema vs. fatty infiltration, the muscles most affected in each compartment, using the Mercuri score [11, 12].

Results

Phenotypic presentation and clinical investigations

Family A (FA)

An Argentinean family originally from the province of Buenos Aires with Italian ancestry. There were 10 affected family members over 3 generations (Fig. 1). All affected individuals had a normal early motor development. The age at onset ranged from 38 to 48 years. The initial symptoms were proximal lower limb weakness in two patients (FA.II-6, II-9) associated with distal lower limb weakness in one of them (FA.III-1). Scapular winging and anterior thigh atrophy were common findings in all patients (Fig. 2). Other symptoms and signs were muscle cramps (FA. I-6, II-6 and II-10) and early onset cataracts (FA. I6, II-4, II-6 and II-10). No additional clinical information was available from the deceased relatives.

FA.III-1

A 56-year-old male with asymmetric distal lower limb weakness from the age of 38, unable to walk on heels. Muscle weakness progressed to the proximal lower limb muscles, and to the shoulder and arm muscles. This resulted in frequent falls. Later on, he developed limitations of finger and toe flexors. At present he is unable to raise his arms, has marked bilateral scapular winging (Fig. 2b), symmetrical quadriceps atrophy and walks with a cane. No cataracts were observed. CK levels were variably increased at several determinations, with values between 677-1800 IU/l [normal values (nv): 55–170 IU/l]. Cardiovascular and pulmonary evaluation was unremarkable. He had two non-affected brothers that are deceased. His deceased father developed scapular winging during adulthood and cataracts at 70 years of age.

FA.II-6

A 68-year-old woman whose first symptom was early onset cataracts revealed at the age of 42 years. By the age of 48, she developed proximal lower limb weakness, progressive difficulty in climbing stairs and frequent falls. Later on, the weakness progressed to distal muscles with movement limitation affecting first the toes and later the finger flexors and small hand muscles. Scapular winging was also present (Fig. 2e).Currently she has both proximal and distal lower limb weakness (MRC 3/5), walks with a cane, and experiences frequent cramps in her feet, legs, and hands. She had a medical history of hypothyroidism controlled with medication. The CK levels were slightly elevated at 300 IU/l (nv: 30–135 IU/l). The EMG showed a "myopathic" pattern consistent with a distal involvement with normal nerve conduction studies (NCS).

FA. II-9

A 59-year-old man, started at the age of 38 with proximal and later on distal muscle weakness. Similar to the other patients he presented with limitation of toes and later finger flexors; and scapular winging (Fig. 2a). He has a normal cardiovascular evaluation; reduced forced vital capacity with ventilatory insufficiency.

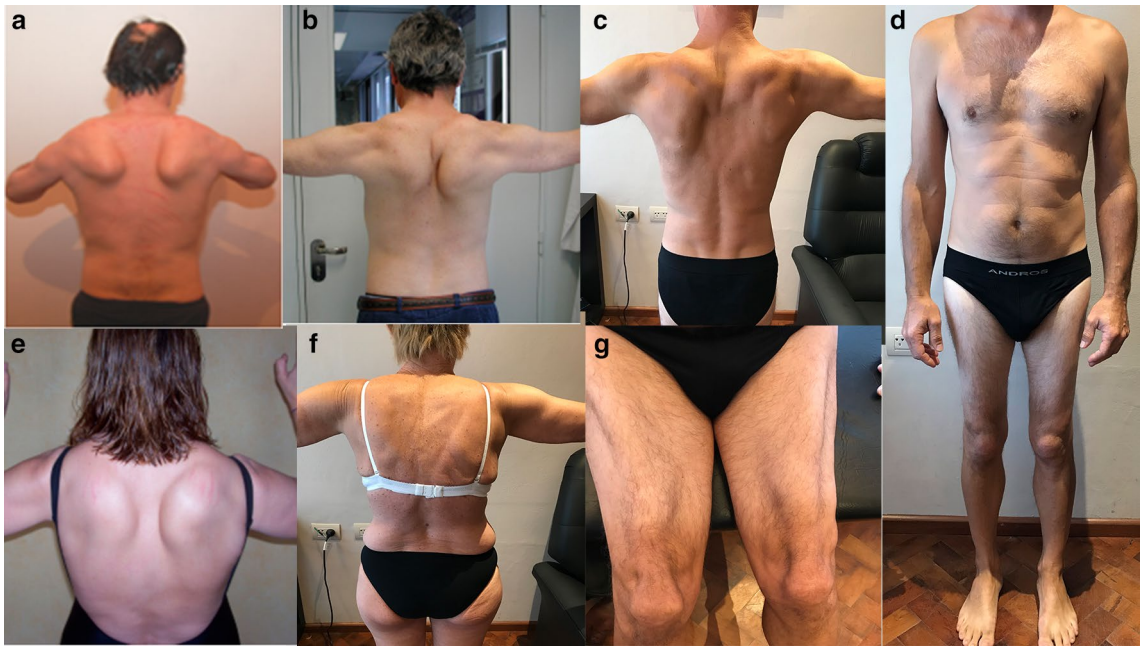

Fig. 2 Scapular winging and vastus atrophy are common clinical findings. Scapular winging was a common finding in patients: FA.II-9 (**a**), FA.III-1 (**b**), FB.III-1 (**c**), FA.II-6 (**e**) andFB.II-1 (**f**). Severe thigh atrophy was present in all patients (patient B.III-1) (**d**, **g**). In case B.II-1 (**f**) although she carries the mutation, slight symptoms were present. Marked atrophy of vastus muscles were observed with preservation of the rectus femoris (Patient B.III-1) (**g**)

Family B (FB)

An Argentinean family from the province of Entre Rios, with French-Basque ancestry with 5 affected members over three generations (Fig. 1). Age at onset varied from 41 to 63-year-old with progressive proximal lower limb muscle weakness. There was no history of early onset cataracts or restriction of finger and toe flexion with reduced range of movement in the interphalangeal joints.

FB.III-1

A 46-year-old man, semi-professional cyclist, started to experience progressive weakness and wasting of the quadriceps from the age of 41. He has difficulty standing from the crouched position, has occasional unexplained falls and some difficulty climbing stairs. Cardiopulmonary examination was normal. The maternal grandfather and three great uncles were affected by a similar, late onset, muscle condition. At the time of his first examination at age 41, he had no shoulder girdle or upper arm muscles weakness but a mild bilateral scapular winging; prominent atrophy of the vastus lateralis and medialis and a striking preservation of the rectus femoris (knee extension: MRC power grade 4-/5) (Fig. 2d,g). Atrophy of the posterior thigh compartment was noticed as well as abdominal weakness. Pelvic girdle weakness was present with hip adduction and abduction being compromised (MRC power grade 4+/5). Muscle strength was preserved in the lower legs and there was calf hypertrophy. Five years after the first evaluation a more pronounced scapular winging (Fig. 2c) and shoulder weakness with no lower limb distal weakness was observed. No finger or toes flexion limitation was present. The CK levels were mildly elevated (862 U/l; nv: 55–170 UI/l) and the electromyography was consistent with a "myopathic" pattern.

FB.II-1 case

The mother of the proband, a 65-year-old woman, showed a slight weakness of the vastus muscles (Knee extension MRC 4+/5), mild and asymmetrical scapula displacement (Fig. 2f) and lumbar scoliosis. The patient reports occasional lower limb cramps and for the last 3 years some difficulties climbing stairs. The CK level was normal. There is no history of early onset cataracts.

Muscle biopsies

Histochemistry

Quadriceps muscle biopsies from patients FA.III-1, FA.II-6, FA.II-9, FB.III-1 at the age of 42, 55, 40 and 41 years-old, respectively, showed the presence of rimmed vacuoles, autophagic vacuoles and cytoplasmic bodies (Fig. 3). There

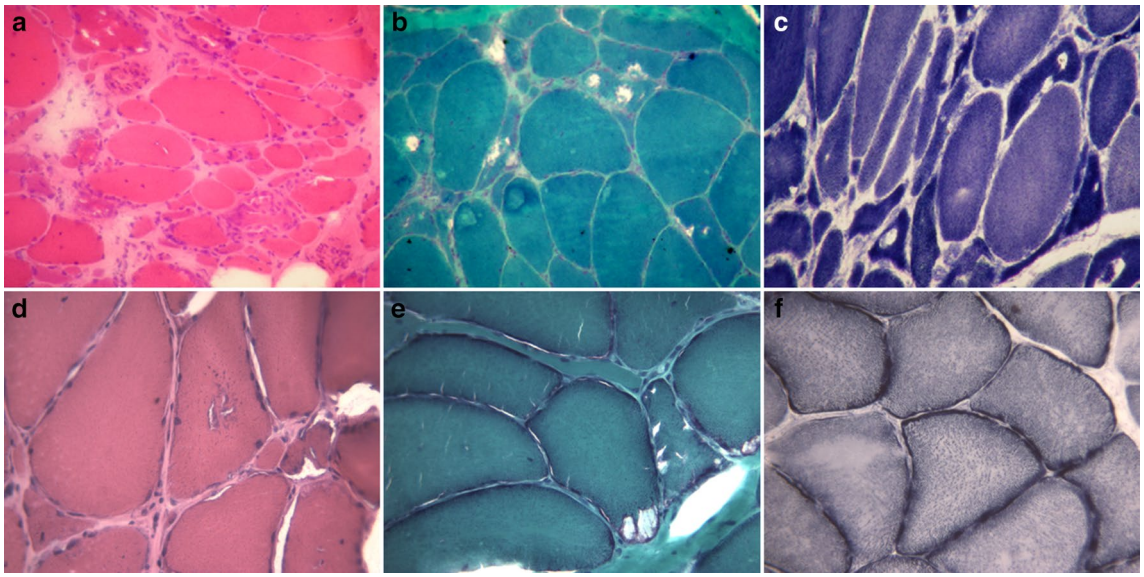

Fig. 3 Histochemistry. Frozen muscle sections from patients: FA. III-1 (**a**–**c**) and FB. III-1 (**d**–**f**). Marked variability of fibre size with some atrophic fibres. Numerous rimmed vacuoles and abundant autophagy lesions were observed in many fibres: **a** (HE), **b** (GT), **d** (HE) and **e** (GT). Vacuoles and some areas of disorganized structure were also detected with oxidative techniques: c and f (NADH-TR)

was variability in muscle fibre size with the presence of atrophic fibres and a few pyknotic nuclear clumps. There was a moderate increase in the endomysial/perimysial connective tissue and in one case, some necrotic/regenerating fibres were observed.

Immunohistochemistry (IHC)

Dystrophin; lamininalpha-2; alpha, beta, gamma, and delta sarcoglycans labelling was preserved. ForFA.II-9: Some of the vacuoles were labelled with antibodies for dystrophin, thus confirming the presence of sarcolemma membranes in the vacuoles as previously described. IHC study for desmin, myotilin, and αB-crystallin did not show any specificity, with only an inconsistent and unspecific immunostaining of a few degenerating fibres. Some vacuoles were labelled with the antibodies against C5b9 and dystrophin and a good number of muscle fibres presented accumulations of P62.

Ultrastructural study

Electron microscopy studies were performed in three muscle biopsies (FA.III-1,FA.II-6 and FB.III-1) (Fig. 4). All biopsies showed marked autophagic changes, particularly in patients FA.III-1 and FB.III-1 and less prominent in patient FA.II-6. We could observe numerous vacuoles containing membranous remnants, myeloid bodies, and cytoplasmic bodies (Fig. 4b, c, f). The myofibrils were regularly dissociated by amorphous material from the autophagy debris. Cytoplasmic areas with tubulofilamentous inclusions, a structural marker of rimmed vacuoles, were detected in some muscle fibres (Fig. 4a, d). Additionally, remaining segments of disrupted sarcomeres were present in the disorganized regions of the fibres, and small areas of dispersed filamentous bundles of Z-disc origin were also noted (Fig. 4e). Of note that, neither granular filamentous aggregates nor nuclear tubulofilamentous inclusions were observed in these muscle biopsies.

Genetic analysis

By exome sequencing, we identified the same heterozygous *HNRNPDL* mutation in both families. This missense mutation, c.1132G>C p.(Asp378His), affects a conserved amino acid encoded by exon 6 of *HNRNPDL*. The mutation was not listed in gnomADor in our in-house database containing over 1500 exomes and was previously reported as causing LGMDD3. In family A, the presence of the *HNRNPDL* mutation in the patients and its absence in the healthy family members was confirmed by Sanger sequencing. In F.B, *HNRNPDL*: c.1132G>C p.(Asp378His) was found in the index patient as well as the mother, whereas the father and the healthy brother did not harbour the mutated allele.

MRI findings

MRI findings showed in the three cases analysed at the thighs, a selective pattern involving heavily adductor magnus and vastus muscles with preservation of rectus femoris and adductor longus (Fig. 5). Lower distal leg involvement

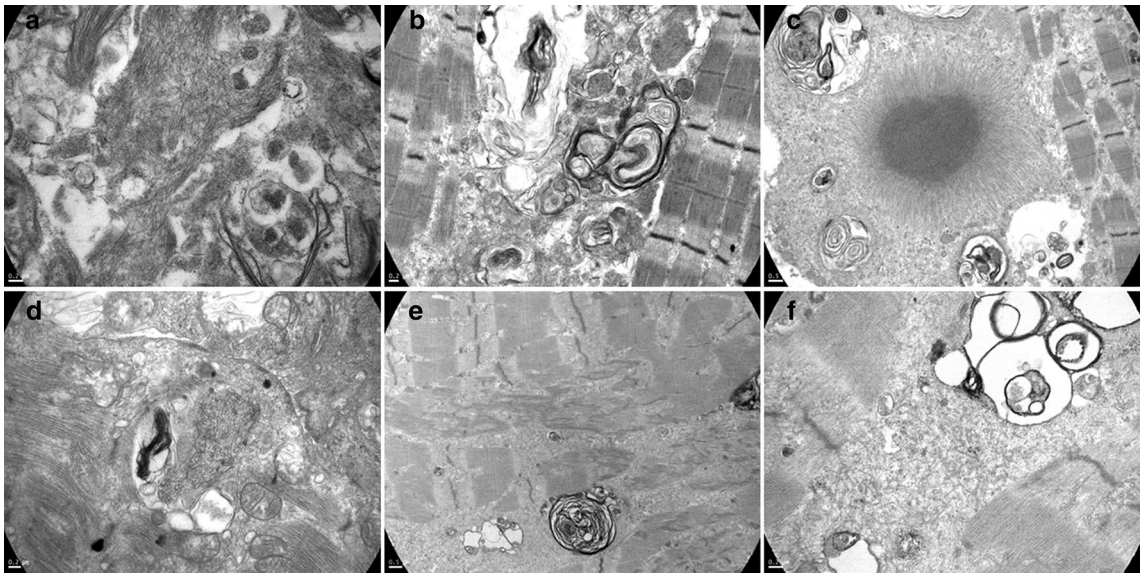

Fig. 4 Ultrastructural findings. Longitudinal muscle sections from patients: FA. III-1 (**a**–**c**) and FB. III-1 (**d**–**f**). The autophagic vacuoles containing membranous remnants, myeloid bodies and some glycogen granules (**b**, **f**) are observed among the myofibrils. Cytoplasmic bodies (**c**) and few zones with spread filamentous bundles of Z-disc material (**e**) were also noted. Areas with tubulofilamentous inclusions, a structural marker of rimmed vacuoles, were detected in some muscle fibres (**a**, **d**)

was present only in one patient showing replacement of anterior compartment and soleus muscles.

FA. II-6

Muscle MRI shows a diffuse, symmetric, fatty infiltration of the gluteus minimus and ilio-psoas muscles; moderate fatty infiltration of the gluteus maximus predominantly on the right and of the tensor fascia lata with relative sparing of gluteus medium muscles. At the thighs, on the anterior compartment, a selective involvement of vastus medialis, intermedius and lateralis, sartorius, adductor magnus, with relative sparing of the rectus femoris and adductor longus muscles were observed (Fig. 5e). On the posterior compartment there was a severe involvement of the semitendinosus with relative sparing of the semimembranosus and long head of biceps femoris. At the lower legs the tibial anterior, extensor hallucis and soleus were affected (Fig. 5f).

FB.III-1

Thigh MRI showed severe fatty infiltration of all vastus muscles, sartorius and adductor magnus with relative sparing of gracilis, biceps femoris, semitendinosus, and semimembranosus muscles. Preservation of rectus femoris and adductor longus were observed with hypertrophy of rectus femoris (Fig. 5c). At the lower leg there was no evident muscle involvement (Fig. 5d).

FB. II-1

Severe fatty infiltration was observed in the vastus lateralis, adductor magnus and sartorius muscles with relative sparing of rectus femoris and the adductor longus (Fig. 5a). Semitendinosus, semimembranosus and biceps femoris were moderately affected. At the lower leg level, no evident involvement was detected (Fig. 5b).

Comparative MRI studies

Comparative MRI studies from both FA. II-6 and FB.III-1 patients were performed with an interval of 12 years and 4 years, respectively (Fig. 6).

Discussion

LGMDD3 HNRNPL-related is caused by heterozygous mutations in the *HNRNPDL* gene. Until now, only three families were described worldwide: a Brazilian-Caucasian, a Uruguayan, and a Chinese family [2-4]. The missense mutations in *HNRNPDL* affected the same aminoacid in all families although the transition is different: p.(Asp378Asn) in the Brazilian and Chinese families and p.(Asp378His) in the Uruguayan family. The description of several families carrying the mutation in the same amino acid could define a mutational hotspot.

This study describes two unrelated Argentinian families with European ancestry (Italian/French-Basque) with

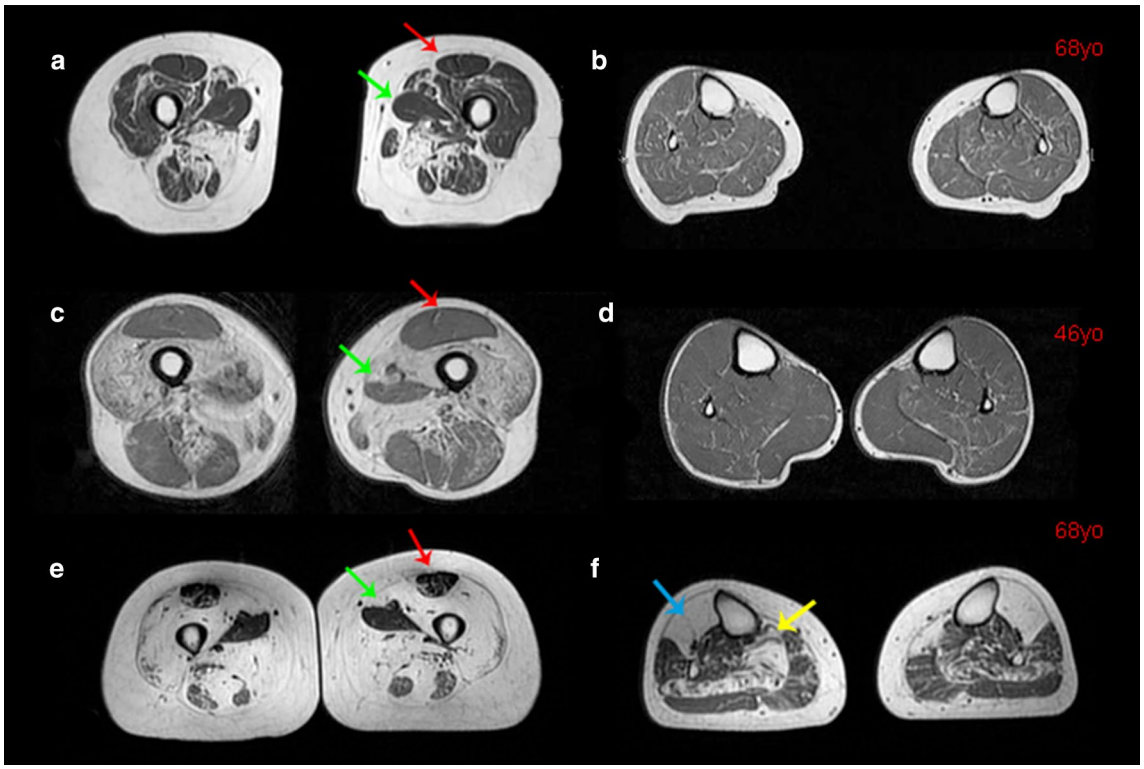

Fig. 5 MRI reveals a specific muscle pattern at the thigh. T1 weighted MRI sections shows preservation of adductor longus and rectus femoris as a common finding in all patients, even in different stages of the disease. **a** Thigh MRI Patient FB.II-1 performed at age 68; severe fatty infiltration was observed on the vastus lateralis, adductor magnus and sartorius muscles with relative sparing of rectus femoris (arrow) and the adductor longus (arrow). Semitendinosus, semimembranosus and biceps femoris were moderately affected. **b** At the leg level no evident involvement was observed. **c** Thigh MRI in patient FB.III-1 performed at age 46 showed severe fatty infiltration of vasti muscles, Sartorius and adductor magnus with relative sparing of gracilis, biceps femoris, semitendinosus and semimembranosus muscles. Preservation of rectus femoris and adductor longus were observed with hypertrophy of rectus femoris. **d** At the leg level no evident involvement was observed. **e** Muscle MRI in patient FA.II-6 performed at 68-year-old. At the thigh level, almost complete fatty replacement is observed with selective involvement of vastus medialis, intermedius and lateralis, sartorius and adductor magnus with relative sparing of the rectus femoris and adductor longus. In the posterior compartment, severe involvement of semitendinosus was seen, with relative sparing of semimembranosus and long head of biceps femoris. **f** At the legs, tibial anterior (arrow), extensor hallucis and soleus (arrow) were also affected

LGMDD3 HNRNPDL-related, histologically characterized by marked autophagy with numerous rimmed vacuoles associated with the p.(Asp378His) dominant *HNRNPDL* mutation. Moreover, we provide new clinical, histopathology and ultrastructural findings, as well as evidence of a selective muscle MRI involvement which seems to be specific in any stages of the disease, even in oligo-symptomatic patients.

In our two families, the disease manifested with two different initial phenotypes; a typical late onset limb girdle proximal weakness with atrophy of the thighs, and a distal asymmetric lower limb ankle dorsiflexion weakness. Whereas limitation of fingers flexors was a late manifestation in all members of one of our families, early onset cataracts were present in only four patients. Despite the variable presentation in both families, scapular winging was a regular clinical finding in all affected patients. The presence of a 65 years-old oligo-symptomatic patient reinforces previous descriptions regarding variable clinical severity of the disease. In our cohort, as in previous reported series, the myopathy usually manifested as a late onset slowly progressive disorder, the age of onset ranging from 38 to 63 years. Normal cardiac and respiratory assessments in most of the studied patients could suggest that cardiac and respiratory insufficiency may not be a feature of this condition, however, the oldest patient in this series presents a reduction of the FVC over time with a pattern that may suggest the diaphragm is compromised. We, therefore, suggest that the respiratory function should be monitored in this condition. Some clinical similarities can be established between LGMD D3 HNRNPDL-related and myopathies associated with mutations in other genes coding for other heterogeneous nuclear ribonucleoproteins (hnRNPs) such as hnRNPA1. Rimmed vacuolar myopathy has been documented with mutations in hnRNPA1, causing a similar autosomal dominant, late onset

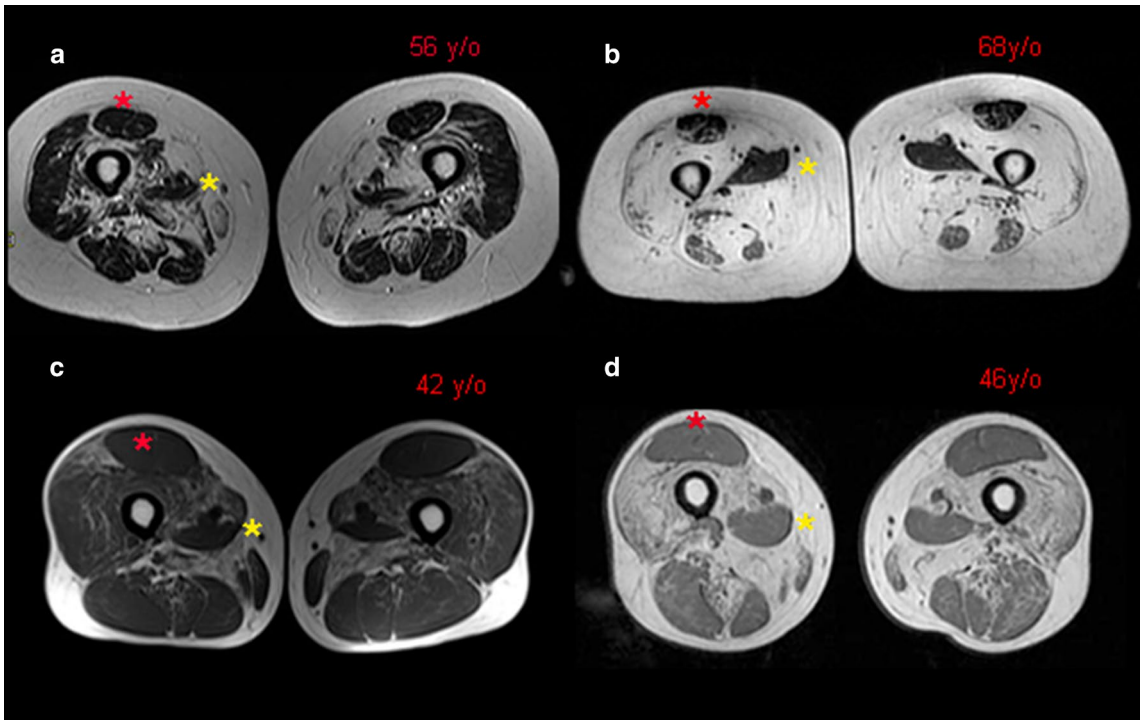

Fig. 6 Comparative thigh MRI in two different patients. T1 weighted comparative thigh MRI performed in patient FA.II-6 (56-year-old and 68-year-old) (**a**, **b**) and FB.III-1 (42-year-old and 46-year-old) (**c**, **d**) which illustrates selective sparing of rectus femoris (asterisk) and adductor longus (asterisk) through the years with marked degeneration of vasti. Posterior compartment muscles in case FA.II-6 were severely affected with the exception of semimembranosus and biceps femoris whereas in FB.III-2 are less affected

myopathy with distal or proximal lower limb onset and no cardiac or respiratory involvement [9, 10, 13].

Our MRI findings allow us to postulate a specific and pathognomonic pattern of involvement of the thighs with preservation of the rectus femoris and adductor longus, even at late stages of the disease (Figs. 5, 6).These findings are in accordance with the previous description by Sun et al. (2019), showing a strikingly similar muscle MRI pattern. Vastus muscles seem to be early and particularly affected and probably may correlate with the early falls of the patients. In contrast, no leg-specific muscle involvement was observed. A similar muscle MRI pattern, with preservation of the rectus femoris was described previously in MSPs like hnRNPA1 and hnRNPA2B1 associated diseases [9]. The reason for the selective involvement of some particular muscles with relatively sparing of others remains unclear. We could speculate that a possible association of myotoxicity with the levels of mutant HRNPNDL in different muscles might be related with the selective muscle involvement, or some muscles being more dependent on correct mRNA-processing than others. Selective muscle involvement is not a specific finding of LGMD D3 HNRNPDL-related, but rather a common finding in muscular dystrophies. Previous reports demonstrated significant variations in isoform and gene expression levels in anatomically different muscles as one of the possible explanations of this discrepancy [14]. Other factors remain to be clarified.

The muscle biopsy studies allowed us to demonstrate a particular morphological pattern characterized by fibres harbouring numerous autophagic rimmed vacuoles and cytoplasmic bodies. Electron microscopy, performed for the first time in HNRNPDL-related LGMD D3, demonstrated autophagic changes with numerous vacuoles containing membranous remnants, myeloid bodies, besides cytoplasmic bodies and some filamentous material. Myofibrils in dissolution were separated by amorphous autophagic debris material (Fig. 4).

The morphological and clinical aspects of the *HNRNPDL*-related myopathy, suggest that this entity should be included in the group of the "autophagic vacuolar myopathies" (AVM) instead of the LGMD. Of note, the cases previously reported of *HNRNPDL*-related muscular dystrophy in the literature did not include electron microscopy studies and the morphological description was very limited. These facts have led to the classification of this entity as an autosomal dominant muscular dystrophy mostly based on the clinical phenotype. With the new findings in electron microscopy that we report here, we postulate that the histological phenotype is compatible with an AVM and we suggest that the classification of this entity should be reconsidered.

HNRNPDL belongs to the subfamily of the heterogeneous nuclear ribonucleoproteins (hnRNPs) essential for the maturation of newly formed heterogeneous nuclear pre-mRNA into mRNA and the transport of mRNA. They are key proteins in the RNA metabolism and many hnRNP genes are known to cause degenerative diseases in particular with mutations in the prion-like domains (PrLD). Mutations in the PrLD of hnRNPA1 and A2B1 cause MSP [10]. Mutations in the PrLD of TIA1 cause rimmed vacuolar distal myopathies [15]. Mutations in the PrLD increase the tendency to self-aggregation.

In conclusion, HNRNPDL-related LGMD D3 results in a wide range of phenotypes from the classic proximal form of LGMD to a more distal phenotype. Codon 378 of HNRNPDL gene can be considered a mutation hotspot for HNRNPDL-related myopathy. Moreover, cataracts and limited fingers flexion may not be uniform findings. On the other hand, scapular winging is an important clinical key sign with similarity to VCP mutated MSP myopathy. The preservation of rectus femoris and adductor longus muscles is a common MRI feature at all stages of the disease. Pathologically the disease can be classified among the autophagic rimmed vacuolar myopathies as with the other MSP myopathies.

Acknowledgements This work has been financially supported by Association Françaisecontre les Myopathies (AFM-Telethon), Association Institut de Myologie (AIM), Assistance Publique-Hôpitaux de Paris (AP-HP), Institut National de la Santé et de la RechercheMédicale (INSERM), Sorbonne Université, Centre National de la RechercheScientifique (CNRS), University of Strasbourg, the France Génomique National infrastructure funded as part of the Investissementsd' Avenir program managed by the AgenceNationale pour la Recherche (ANR-10-INBS-09) and by Fondation Maladies Rares within the frame of the "Myocapture" sequencing project, the Fondation pour la RechercheMédicale.

Compliance with ethical standards

Conflicts of interest All authors have reviewed the manuscript. Authors have no conflict of interest to declare.

Ethical standards The study was conducted after receiving written informed consent from patients in accordance to the declaration of Helsinki.

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