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ORIGINAL COMMUNICATION

Bethlem myopathy: a series of 16 patients and description of seven new associated mutations

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Abstract

Background Bethlem myopathy represents the milder phenotype of collagen type VI-related myopathies. However, clinical manifestations are highly variable among patients and no phenotype-genotype correlation has been described. We aim to analyse the clinical, pathological and genetic features of a series of patients with Bethlem myopathy, and we describe seven new mutations.

Methods A series of 16 patients with the diagnosis of Bethlem myopathy were analyzed retrospectively from their medical records for clinical, creatine kinase (CK), muscle biopsy, and muscle magnetic resonance (MRI) data. Genetic testing was performed through next-generation sequencing of custom amplicon-based targeted genes panel of myopathies. Mutations were confirmed by Sanger sequencing.

Results The most frequent phenotype consisted of proximal limb weakness associated with interphalangeal and wrists contractures. However, cases with isolated contractures or isolated myopathy were found. CK levels did not correlate with severity of the disease. The most frequent mutation was the COL6A3 variant c.7447A>G, p.Lys2486Glu, with either an homozygous or compound heterozygous presentation. Five new mutations were found in COL6A1 gene and other two in COL6A3 gene, all of them with a dominant heritability pattern. From these, a new COL6A1 mutation (c.1657G>A, p.Glu553Arg) was related to an oligosymptomatic phenotype with predominating contractures in the absence of weakness and a normal muscle MRI. Finally, the most common COL6A1 mutation reported to date that leads to an Ullrich phenotype (c. 868G>A, p.Gly290Arg), has been found here as Bethlem presentation.

Conclusions Manifestations of Bethlem myopathy are quite variable, so either contractures or weakness may be lacking, and no phenotype-genotype associations can be brought.

Keywords Bethlem myopathy · Joint contractures · Proximal muscular weakness · COL6 genes · Collagen type VI-related myopathies

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Introduction

Collagen type VI-related myopathy (COL6 myopathy) includes a wide spectrum of muscular dystrophies caused by a defect in the genes encoding the collagen VI α-chains (COL6A1, COL6A2, COL6A3). Collagen VI is an extracellular matrix protein found in most tissues. Its absence or aberrant formation results in muscle dystrophy with connective tissue involvement. Thus, it represents a genetically and phenotypically heterogeneous group of disorders characterized by variable muscle weakness, hypotonia, distal hyperlaxity, joint contractures and skin changes.

In this wide clinical spectrum, the most severe form is known as Ullrich muscular dystrophy, characterized by congenital muscle weakness that leads to impossibility to acquire ambulation or to the loss of this ability before adulthood. By contrast, in the other clinical extreme, we find the so-called Bethlem myopathy, that includes a high diversity of milder forms with variable age-at-onset and slowly progressive muscle weakness and/or joint contractures [1]. With the diffusion and improvement of the genetic studies, several other intermediated phenotypes caused by collagen VI genes mutations have been described [2, 3] but their boundaries are not clear at all. Genotype–phenotype association or early predictors for progression have not been yet identified, although the presence of multiple joint contractures seems to predict rapid deterioration [4, 5].

Although Bethlem myopathy is mostly inherited as an autosomal dominant disease, recessive patterns have also been described [6, 7].

In this study, we present the clinical, pathological and genetic features of 16 patients with Bethlem myopathy, and we describe 7 new mutations related to this disease.

Methods

Patients and data

The study subjects were recruited retrospectively from the database of the Neuromuscular Disorders Unit of *Hospital Universitario 12 de Octubre*, in Madrid, a national reference centre of rare neuromuscular diseases. All the patients included in the series were diagnosed of Bethlem myopathy between the years 2012 and 2018, according to clinical, radiological and genetic results. Written informed consent was obtained from all patients at the beginning of the follow-up at the Neuromuscular Disorders Unit according to the Ethics Committee requirements. Clinical records were reviewed for demographic and clinical data (Table 1).

Concerning complementary studies (Table 2), creatine kinase (CK) levels were determined at least once and the highest level was pointed. All the patients were submitted to muscle magnetic resonance imaging (MRI) in a 1.5 T system. A muscle biopsy was obtained from 10 out of the 16 patients and assessed at the light microscopic using the regular staining methods. The presence of increased endomysial connective tissue and fatty replacement allowed categorizing the sample as a dystrophic muscle. Other atypical histological features were also pointed, if present. Collagen VI immunohistochemical analysis on muscle samples, using collagen IV as an internal control, were performed in 6 out of 16 patients.

Genetic analysis

Genetic testing was performed through next-generation sequencing (NGS) of custom amplicon-based targeted 59 genes panel known to be associated to muscular dystrophies and other myopathies (Ion-PGM platform; Thermo Fisher Scientific, Carlsbad, CA, USA). The pathogenicity of novel mutations was assessed depending on the following criteria: (1) the functional importance of the affected domain of the protein; (2) the nature of the mutation (nonsense or insertion/deletion vs missense); (3) its absence in variants' databases (EVS, 1000G); (4) in silico bioinformatics analyses using CONDEL [8] and HSF 3.1 [9]; (5) following American College of Medical Genetics and Genomics (ACGM) consensus criteria for the interpretation of sequence variants; (6) cosegregation with the disease in the available relatives. All mutations detected were confirmed by Sanger sequencing.

Results

Clinical features

Sixteen patients met the exposed criteria for Bethlem myopathy, ten males and six females (Table 1). The medium age at diagnosis was 48 years (range 19–72 years). Six patients started with symptoms at childhood (37%) and five at adulthood (31%), the other five had an uncertain beginning of the disease due to the scarcity of clinical manifestations. Weakness was present in 13 out of 16 patients (81%), ranging from mild to severe. It mainly affected proximal limbs, with special involvement of quadriceps muscles, and it associated cervical involvement in five cases (31%) and distal limb weakness (which comprised wrist and finger extension in upper limb or ankle dorsiflexion and toes extension in lower limb) in other five patients (31%). Only one patient (11A) showed distal weakness without proximal involvement. Gait impairment was observed in five cases (31%) and

Table 1 Clinical features

Family	Case	Genre	Age[a]	Age at onset	Familiar antecedents of muscle disease	First symptom	Weakness	Contractures	Scoliosis	Cutaneous alterations	Ventilatory insufficiency
1	1A	F	43	Childhood	No	Floppy newborn	Facial, cervical, proximal UL and LL	Wrists, interphalangeal, ankles	Yes	No	NIV
2	2A	M	49	Unknown	Brother (Steinert disease)	Weakness	Proximal UL and LL	Interphalangeal, ankles	No	No	No
3	3A	M	72	Childhood	No	Clumsiness and contractures	Cervical, proximal UL and LL	Elbows, wrists, interphalangeal, ankles	No	Cigarette paper scars	NIV
4	4A	M	31	Unknown	Index case	HyperCKemia	No	Interphalangeal, ankles	No	No	No
	4B	M	33	Unknown	Brother	Asymptomatic	No	Interphalangeal, ankles	No	No	No
	4C	F	61	Unknown	Mother	Asymptomatic	Cervical, fingers, proximal LL	Interphalangeal, ankles	Yes	No	No
5	5A	M	42	35 years	No	Weakness	Proximal UL and LL	Interphalangeal, knees	No	No	No
6	6A	M	65	Childhood	No	Weakness and contractures	Proximal and distal UL, proximal LL	Elbows, wrists, interphalangeal	No	Hyper-keratosis	No
7	7A	M	59	50 years	No	Weakness	Facial, cervical, proximal UL and LL	Interphalangeal	No	Cheloids	No
8	8A	F	27	Childhood	No	Weakness	Cervical, proximal UL and LL	Elbows, ankles	No	No	Reduced MEP
9	9A	M	42	Childhood	Index case	Weakness	Proximal LL	Interphalangeal, ankles	No	No	No
	9B	M	48	Unknown	Brother	HyperCKemia	No	Interphalangeal, ankles	No	No	No
10	10A	F	63	40 years	Index case	Weakness	Distal UL and proximal LL	No	No	No	No
	10B	F	67	64 years	Sister	Weakness	Distal UL and proximal LL	No	No	No	No
	10C	M	55	52 years	Brother	Weakness	Proximal UL and LL	Interphalangeal, wrists	No	Cheloids	No
11	11A	F	19	Childhood	Father (probable Bethlem disease[b])	Weakness and contractures	Distal LL	Interphalangeal, ankles	No	No	No

ALS amyotrophic lateral sclerosis, *LL* lower limbs, *MEP* maximal expiratory pressure, *NIV* non invasive mechanical ventilation, *UL* upper limbs

[a]Age at diagnosis

[b]Deceased father with contractures

Table 2 Complementary studies

Family	Case	CK (IU/L)	Muscle MRI	Muscle biopsy	GEN	Nucleotide change	Protein change	Haplotype	AGCM classification		First description
1	1A	< 170	Typical	Lobulated fibers	COL6A1	c.868G>A	p.Gly290Arg	Heterozygous	PS1, PM2/PM5, PP3/PP5	Pathogenic	Lampe et al. [12]
2	2A	450	Typical	Dystrophy	COL6A1	c.739-2A>G	Splicing (exon skipping)	Heterozygous	PP3, PVS1, PM2	Pathogenic	Deconinck et al. [21]
3	3A	< 170	Typical	Dystrophy	COL6A1	c.505T>G	p.Cys169Gly	Heterozygous	PP1/PP3, PM2	Uncertain significance	New
4	4A	2800	Unilateral medial gastrocnemius peripheral infiltration	Minimum non-specific changes	COL6A1	c.1657G>A	p.Gly553Arg	Heterozygous	PP1/PP3, PM2	Uncertain significance	New
	4B	570	Normal	–							
	4C	< 170	Normal	–							
5	5A	1200	Typical	Dystrophy, rimmed vacuoles	COL6A1	c.1398+2T>C	Splicing	Heterozygous	PP3, PVS1, PM2	Pathogenic	New
6	6A	< 170	Typical	–	COL6A1	c.1002G>A	p.K334K	Heterozygous	PP3, PM2	Uncertain significance	New
7	7A	230	Typical	Dystrophy	COL6A2	c.2098G>A	p.Gly700Ser	Heterozygous	PP3/PP5, PS1, PM2	Likely pathogenic	Lampe et al. [12]
8	8A	800	Typical	Dystrophy	COL6A3	c.7447A>G	p.Lys2483Glu	Heterozygous	PP3	Uncertain significance	Briñas et al. [1])
						c.8540_8540delA	Frame-shift deletion	Heterozygous	PVS1, PM2	Likely pathogenic	New
9	9A	4000	Typical	Dystrophy, rimmed vacuoles	COL6A3	c.7447A>G	p.Lys2483Glu	Homozygous	PP1/PP3	Uncertain significance	Hunter et al. [13]
	9B	1000	Typical	–							
10	10A	250	Typical	Dystrophy	COL6A3	c.6320_6322del	In-frame deletion	Heterozygous	PP1, PM2, PM4	Uncertain significance	New
	10B	< 170	Typical	Dystrophy							
	10C	220	Typical	–							
11	11A	830	Typical	–	COL6A3	c.7447A>G	p.Lys2483Glu	Homozygous	PP3	Uncertain significance	Hunter et al. [13]
					COL6A1	c.2435-2A>G	Splicing	Heterozygous	PVS1, PM2, PP3/PP5	Pathogenic	New

two patients needed technical aids for walking (patients 3A and 10C). All but two patients in this series had contractures (Fig. 1a), being the interphalangeal and ankle joints the most frequently affected. Patients 10A and 10B showed a pattern of distal upper limb and proximal lower limb weakness without contractures. Three patients (18%) had no weakness and interphalangeal and ankle contractures were found to be the sole manifestation of the disease. Characteristic cutaneous alterations, namely cheloids, hyperkeratosis and cigarette paper scars (Fig. 1b), were observed in four patients (25%), and systemic involvement with respiratory symptoms was noted in three cases (18%). In this sense, patients 1A and 3A, who showed both childhood onset weakness, had ventilatory insufficiency, needing non-invasive mechanical ventilation with nocturnal bi-level positive airway pressure (BiPAP) since the age of 40 and 63 years old, respectively. Patient 8A showed reduced maximal expiratory pressure with no needs of ventilatory support.

Eleven patients (69%) showed slight to moderately increased CK levels, which ranged between 220 and 4000 IU/L, whereas the other five had normal CK values (< 170 IU/L). Regarding the muscle MRI, findings were considered "typical" of Bethlem myopathy if concentric involvement of *vastus lateralis* and central high signal in *rectus femoris* were present [10, 11]. Thus, a typical pattern (Fig. 1c, d) was observed in 13 patients (81%), whereas the 3 members of Family 4 showed either a normal image or minimum alterations which did not involve the thigh region. Finally, the muscle biopsy, which was obtained in 10 out of 16 cases (62%), showed mild to moderate dystrophic changes (Fig. 1e), excepting in patient 1A, where only lobulated fibers were found and in patient 4A, where only unspecific changes were observed. "Rimmed vacuoles", in addition to dystrophic changes, were detected in patients 5A and 9A. The dystrophic changes observed in most biopsies appear quite compatible with a collagenopathy, despite collagen VI immunohistochemical reaction properties on muscle samples were normal in all 6 cases where it was performed (Fig. 1f).

Mutation identification in the COL6A genes

COL6A1 gene

Seven different mutations in COL6A1 gene (NM_001848.2) were detected in nine patients of seven different families, all of them with a dominant heritability pattern. From these mutations, we report five that have not been previously described. Two of them led to a single amino acid change: patient 3A carried a heterozygous mutation (c.505T>G, p.Cys169Gly) at the N-terminal location of the protein; and three symptomatic members of Family 4 shared a heterozygous mutation at the triple helix region of the gene (c.1657G>A, p.Glu553Arg). The segregation of the mutation with the disease in this family supports its pathogenicity, despite being classified as uncertain significance variant by AGCM criteria. A third new variant leading to a silent point mutation (c.1002G>A, p.K334K), that was classified as uncertain significance by AGCM classification criteria, was detected in patient 6A. Nevertheless, bioinformatics evaluation of this silent mutation showed that it might inhibit splicing leading to intron retention. Thus, it is considered to affect the accurate pre-messenger RNA (mRNA) splicing either by influencing regulatory elements, leading to

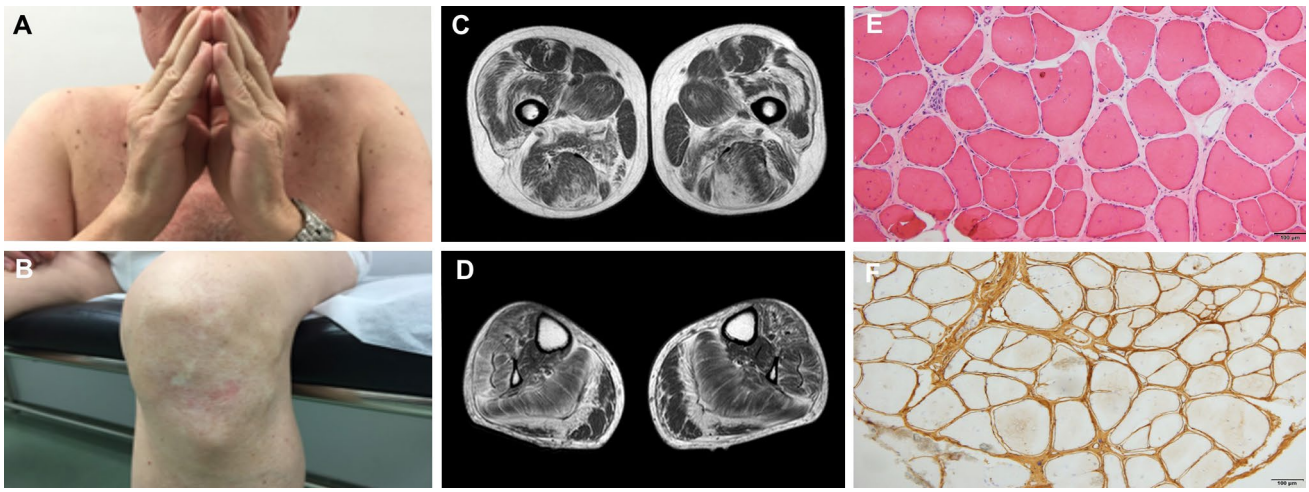

Fig. 1 Characteristic interphalangeal contractures (**a**) and cigarette paper scars (**b**). Typical muscle MRI findings of Bethlem myopathy in T1-weighted sequence showing concentric involvement of *vastus lateralis* and central high signal in *rectus femoris* (**c**), as well as peripheral involvement of medial *gastrocnemius* muscle (**d**). Hematoxylin-eosin staining of muscle biopsy showing moderate dystrophic changes (**e**). Normal collagen VI immunohistochemical staining on muscle biopsy (**f**)

exon skipping, or by creating a new cryptic splice site [9]. Two new intronic mutations in heterozygosis were detected: patient 5A harbored the c.1398+2T>C mutation in intron 20 and Patient 11A had the mutation c.2435-2A>G in intron 35; both were predicted as pathogenic as they alter the WT donor site, thus affecting splicing. However, c.2435-2A>G variant cannot be definitely associated with the disease as far as it was found in a patient (11A) that harbored another mutation in homozygosis at COL6A3 gene, previously associated with Bethlem disease (c.7447A>G, p.Lys2483Glu). Finally, patients 1A and 2A harbored dominant previously described pathogenic mutations.

COL6A2 gene

An already described COL6A2 gene (NM_001849.3) point mutation [12] was detected in heterozygosis in patient 7A (c.2098G>A, p.Gly700Ser), confirming a dominant heritability pattern.

COL6A3 gene

Seven cases from four different families were found to have mutations in the COL6A3 gene (NM_004369.3). Among these, we report two novel variants: Family 10 harbored a heterozygous, in-frame deletion at exon 19 (c.6320_6322del), predicted as uncertain significance since it is located in a Triple-helical region outside a repeat region, but which segregates with the disease in the family. Patient 8A was a compound heterozygote for the already known c.7447A>G, p.Lys2486Glu mutation together with a novel deletion of a nucleotide at exon 39, c.8540_8540delA, which is classified as pathogenic following AGCM criteria as far as it leads to a frame-shift.

Both cases from Family 9 and patient 11A were homozygous for the already described mutation in exon 36 of COL6A3, c.7447A>G, p.Lys2486Glu [13], a single aminoacid change that would lead to missense changes on protein structure and function, although functional studies about this variant are lacking. This variant was the most common mutation detected in our series, and it seems to have a broad distribution at the Iberian Peninsula since patients 8A and 11A, and Family 9 did not share common geographic origin.

Discussion

We describe a series of 16 patients with Bethlem myopathy, belonging to 11 different families. All these patients had a stablished Bethlem myopathy diagnosis based on clinical, radiological and histological features, harboring variants in at least one of the three genes encoding collagen VI that support such diagnosis.

We would like to highlight the typical clinical and complementary features of Bethlem myopathy, mentioning also peculiar findings observed in some patients and families of our series, drawing special attention to the genetic results. In this sense, on the one hand, we aim to describe those mutations which are mentioned here for the first time in the literature. On the other hand, we discuss the possible genotype-phenotype association in some of the patients included in the series in the light of previous reports. However, for several mutations is not possible to discuss such association as far as either only one patient carrying the mutation has been reported or the clinical picture of the already described mutation is not detailed in the literature.

Clinical aspects

The most frequent phenotype of patients with Bethlem myopathy consists in proximal limb weakness, associated with interphalangeal and wrists contractures, which is a feature that should help raising the diagnostic suspicion of a collagenopathy. Nevertheless, affected patients can show contractures as the sole manifestation of the disease in absence of muscular weakness (Family 4) and others may have only muscle weakness, lacking contractures (patients 10A and 10B). So, the presence of contractures is typical but not obligate for the diagnosis of Bethlem myopathy. These two sisters had a very intriguing phenotype, not only due to the absence of contractures but also regarding the special muscular weakness distribution, with distal upper limbs and proximal lower limbs involvement, which has not been observed in any other patient of the series. Remarkably, they had a quite different phenotype compared to their brother (10C), who showed contractures, cheloids and more severe manifestations with gait impairment and proximal upper limb weakness. These three patients showed the typical pattern of collagen VI deficiency on muscle MRI.

Regarding the complementary tests, in consonant with previous literature [14–16], CK levels may be normal or slight to moderately increased. Two-thirds of our patients showed elevated CK levels without clear clinical correlation. Among the four patients showing normal levels (1A, 4C, 6A, 10B), there is a high clinical variability regarding both disease onset and predominant manifestations (either contractures or muscle weakness). On the other end, among the patients showing moderately increased CK levels, several of them have not muscle weakness (i.e. 4A, 9B). We conclude then that CK levels do not seem to correlate with disease onset and clinical severity of Bethlem myopathy.

Although the vast majority of patients with Bethlem myopathy show a typical pathologic muscle MRI, it is important to recognize that some affected patients can have a normal image, as observed in all the components of Family 4, where two of the members had a normal MRI and only the index

case showed slight and not "typical" muscle infiltration. None of the three members of this family complaint about muscle weakness. Conversely, neither showed patient 9B muscle weakness but he had a pathological and typical muscle MRI. In conclusion, although typical findings in muscle MRI can support and guide the diagnosis of Bethlem myopathy, a normal radiological study does not allow ruling out this entity.

Although not always present, the muscle biopsy showed dystrophic changes in the large majority of cases. However, collagen VI immunohistochemical analysis on muscle samples was normal in all the cases of our series, where it was performed. Then, it does not seem to be useful for the pathological diagnosis of Bethlem myopathy, probably due to milder involvement or a more frequent dominant inheritance pattern, compared to Ullrich myopathy. Finally, collagen VI immunolabeling on cultured skin fibroblasts was not performed, as far as diagnosis was achieved in all cases by congruent clinical, radiological and genetic findings.

Genetics

Most of the cases presented here showed a dominant heritability pattern, with the exception of the COL6A3 c.7447A>G variant. This last mutation turned to be the most frequent one among the series, being detected in three different families from distinct geographical origins. As happens in other genetic diseases, it is very important to perform segregation studies in the families of the index cases, as well as to explore close relatives when possible, since "asymptomatic" individuals may show subtle signs that can implicate an underlying disorder whose diagnosis is relevant mainly for genetic advice.

The phenotype of the novel mutations found at COL6A1 gene was quite variable. The new mutation c.1657G>A was found in three oligosymptomatic relatives (Family 4), who shared a common phenotype consisting of interphalangeal and ankles contractures as the sole clinical manifestation, excepting for the presence of very mild weakness in patient 4C. The index case had been first evaluated because of a hyperCKemia without weakness, whereas his two other relatives were evaluated due to the family history. The muscle MRI of the three patients showed no significant pathological features. Thus, this new mutation in COL6A1 gene seems to be related to a mild clinical and radiological form, and highlights the importance of looking for joint contractures when a collagen disease is suspected. In this sense, a similar but much worse phenotype with predominating contractures has already been described [2] in two siblings with a homozygous COL6A2 mutation, being called "myosclerosis phenotype". Hence the mutation c.1657G>A may represent a benign form of the myosclerosis phenotype.

Among the two mutations in COL6A1 that have already being reported in the literature, the c. 868G>A variant [12] represents the most common mutation in the collagen VI myopathies [17] and it has been related to the more severe extreme of the clinical spectrum of collagen VI myopathies, in the form of Ullrich muscular dystrophy [17–19]. In fact, a pathogenic mutation c.868G>C that causes an identical amino acid substitution has also been reported in Ullrich muscular dystrophy [20]. However, our patient showed a more benign phenotype with childhood onset weakness with contractures and scoliosis but preserving autonomous deambulation at the age of 43 years.

A single case from our series (patient 7A) had a COL6A2 mutation (c.2098G>A), which has been previously found in a family of three affected members [12].

Regarding COL6A3 gene mutations, the newly described c.6320_6322del mutation was found in two siblings with a very similar phenotype, consisting of weakness at distal upper limb and proximal lower limb muscles without associated contractures or cutaneous changes, as well as a third brother who presented a more classical phenotype with proximal limb weakness, contractures and cheloids. Muscle MRI showed typical radiological pattern of Bethlem myopathy in the three cases. Cosegregation of this variant with a clinical picture compatible with Bethlem disease in this family supports the pathogenicity of this new mutation.

The 7447A>G variant was firstly described in compound heterozygosis [1], with a mild phenotype lacking gait abnormalities or contractures. However, the reported case was only 12 years old by the moment she was evaluated, so evolution data regarding muscle weakness and other clinical features are not available. In contrast, the case described by Hunter et al. [13] with compound heterozygosis on this gene had a more severe, Ullrich-like phenotype. We found one case in our series carrying this mutation in heterozygosis together with the new variant c.8540_8540delA, with childhood onset weakness and respiratory involvement.

Previous descriptions of homozygous patients for this mutation [13] are consistent with childhood onset and early weakness with abnormal gait as happened in patients 9A and 11A. However, the same mutation was detected in patient 9B, who was strikingly asymptomatic at mid-age excepting for mild contractures. This finding may indicate that it does not exist a characteristic phenotype associated with this variant, or that further factors could influence on the expression of this genotype. In fact, in patient 11A a novel, presumably pathogenic variant on COL6A1 gene was also detected; however, its role on the symptoms of this patient cannot be stated, as far as the familiar study could not be completed because of the premature death of the father. Taking all together, we believe that these new four cases carrying this mutation and showing a phenotype compatible with Bethlem myopathy support predictions about the pathogenicity of this

variant, with either a recessive or a co-dominant heritability pattern.

A limitation of this study may be the small number of cases included, which could hamper achieving more categorical conclusions. However, given the rarity of this entity, the number of patients presented is to be considered rather high. In this sense, multicentric and prospectively designed studies, including greater number of cases with long-term follow-up, are necessary to deepen the study of this rare entity and to pursue a possible genotype-phenotype association that would allow a more adequate genetic advice for the affected families.

In conclusion, we describe here for the first time seven new mutations in COL6 genes, all them leading to the more benign form of collagen VI myopathies, so-called Bethlem myopathy. Furthermore, some of the already known mutations that were previously reported only in patients with the more severe form, i.e. Ullrich myopathy, have been related in the present study to Bethlem myopathy. Clinical expression of COL6 gene mutations is quite variable, and no phenotype-genotype associations can be brought.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no competing interests.

References

1. Briñas L, Richard P, Quijano-Roy S, Gartioux C, Ledeuil C, Lacène E et al (2010) Early onset collagen VI myopathies: genetic and clinical correlations. Ann Neurol 68(4):511–520
2. Merlini L, Martoni E, Grumati P, Sabatelli P, Squarzoni S, Urciuolo A et al (2008) Autosomal recessive myosclerosis myopathy is a collagen VI disorder. Neurology 71(16):1245–1253
3. Scacheri PC, Gillanders EM, Subramony SH, Vedanarayanan V, Crowe CA, Thakore N et al (2002) Novel mutations in collagen VI genes: expansion of the Bethlem myopathy phenotype. Neurology 58(4):593–602
4. Okada M, Kawahara G, Noguchi S, Sugie K, Murayama K, Nonaka I et al (2007) Primary collagen VI deficiency is the second most common congenital muscular dystrophy in Japan. Neurology 69(10):1035–1042
5. Kim SY, Kim WJ, Kim H, Choi SA, Lee JS, Cho A et al (2018) Collagen VI-related myopathy: expanding the clinical and genetic spectrum. Muscle Nerve 58(3):381–388
6. Foley AR, Hu Y, Zou Y, Columbus A, Shoffner J, Dunn DM et al (2009) Autosomal recessive inheritance of classic Bethlem myopathy. Neuromuscul Disord 19(12):813–817
7. Gualandi F, Urciuolo A, Martoni E, Sabatelli P, Squarzoni S, Bovolenta M et al (2009) Autosomal recessive Bethlem myopathy. Neurology 73(22):1883–1891
8. González-Pérez A, López-Bigas N (2011) Improving the assessment of the outcome of nonsynonymous SNVs with a consensus deleteriousness score, Condel. Am J Hum Genet 88(4):440–449
9. Desmet F-O, Hamroun D, Lalande M, Collod-Béroud G, Claustres M, Béroud C (2009) Human splicing finder: an online bioinformatics tool to predict splicing signals. Nucleic Acids Res 37(9):e67–e67
10. Mercuri E, Lampe A, Allsop J, Knight R, Pane M, Kinali M et al (2005) Muscle MRI in Ullrich congenital muscular dystrophy and Bethlem myopathy. Neuromuscul Disord 15(4):303–310
11. Fu J, Zheng Y-M, Jin S-Q, Yi J-F, Liu X-J, Lyn H et al (2016) "Target" and "Sandwich" signs in thigh muscles have high diagnostic values for collagen VI-related myopathies. Chin Med J 129(15):1811
12. Lampe AK, Dunn DM, von Niederhausern AC, Hamil C, Aoyagi A, Laval SH et al (2005) Automated genomic sequence analysis of the three collagen VI genes: applications to Ullrich congenital muscular dystrophy and Bethlem myopathy. J Med Genet 42(2):108–120
13. Hunter JM, Ahearn ME, Balak CD, Liang WS, Kurdoglu A, Corneveaux JJ et al (2015) Novel pathogenic variants and genes for myopathies identified by whole exome sequencing. Mol Genet Genom Med 3(4):283–301
14. Martins AI, Maarque C, Pinto-Basto K, Negrão L (2017) Bethlem myopathy in a Portuguese patient—case report. Acta Myol 36(3):178–181
15. Fan Y, Liu A, Wei C, Yang H, Chang X, Wang S et al (2018) Genetic and clinical findings in a Chinese cohort of patients with collagen VI-related myopathies. Clin Genet 93(6):1159–1171
16. Suárez B, Lozano-Arango A, Araneda D, Cortés F, Hervias C, Calcagno G et al (2018) Collagen VI related myopathies. When to suspect, how to identify. The contribution of muscle magnetic resonance. Rev Chil Pediatr 89(3):399–408
17. Butterfield RJ, Foley AR, Dastgir J, Asman S, Dunn DM, Zou Y et al (2013) Position of glycine substitutions in the triple helix of COL6A1, COL6A2, and COL6A3 is correlated with severity and mode of inheritance in collagen VI myopathies. Hum Mutat 34(11):1558–1567
18. Pace RA, Peat RA, Baker NL, Zamurs L, Mörgelin M, Irving M et al (2008) Collagen VI glycine mutations: perturbed assembly and a spectrum of clinical severity: collagen VI glycine mutations. Ann Neurol 64(3):294–303
19. Reddy HM, Cho K-A, Lek M, Estrella E, Valkanas E, Jones MD et al (2017) The sensitivity of exome sequencing in identifying pathogenic mutations for LGMD in the United States. J Hum Genet 62(2):243–252
20. Giusti B, Lucarini L, Pietroni V, Lucioli S, Bandinelli B, Sabatelli P et al (2005) Dominant and recessive COL6A1 mutations in Ullrich scleroatonic muscular dystrophy. Ann Neurol 58(3):400–410
21. Deconinck N, Richard P, Allamand V, Behin A, Laforet P, Ferreiro A et al (2015) Bethlem myopathy: long-term follow-up identifies COL6 mutations predicting severe clinical evolution. J Neurol Neurosurg Psychiatry 86(12):1337–1346