ULLRICH CONGENITAL MUSCULAR DYSTROPHY
AND BETHLEM MYOPATHY

Clinical and genetic heterogeneity

Umbertina Conti Reed1, Lucio Gobbo Ferreira2, Enna Cristina Liu3,
Maria Bernadete Dutra Resende2, Mary Souza Carvalho2,
Suely Kazue Marie1, Milberto Scaff4

ABSTRACT - Ullrich congenital muscular dystrophy (UCMD), due to mutations in the collagen VI genes, is an autosomal recessive form of CMD, commonly associated with distal joints hyperlaxity and severe course. A mild or moderate involvement can be occasionally observed. Objective: To evaluate the clinical picture of CMD patients with Ullrich phenotype who presented decreased or absent collagen VI immunoreactivity on muscular biopsy. Results: Among 60 patients with CMD, two had no expression of collagen V and their clinical involvement was essentially different: the first (3 years of follow-up) has mild motor difficulty; the second (8 years of follow-up) never acquired walking and depends on ventilatory support. A molecular study, performed by Pan et al. at the Thomas Jefferson University, demonstrated in the first a known mutation of Bethlem myopathy in COL6A1 and in the second the first dominantly acting mutation in UCMD and the first in COL6A1, previously associated only to Bethlem myopathy, with benign course and dominant inheritance. Conclusion: Bethlem myopathy should be considered in the differential diagnosis of UCMD, even in patients without fingers contractures; overlap between Ullrich and Bethlem phenotypes can be supposed.

KEY WORDS: Ullrich congenital muscular dystrophy, congenital muscular dystrophy, joint hyperlaxity, collagen VI, Bethlem myopathy.

Bethlem myopathy is a dominantly inherited disorder caused by mutations in the three genes of collagen VI, i.e. COL6A1 (21 q22.3), COL6A2 (21 q22.3) and COL6A3 (2 q37)1-4. Although Bethlem myopathy is clinically heterogeneous, most of patients have benign course. The onset may be in the neonatal
period, childhood or adolescence and contractures of fingers, elbows and ankles joints represent a hallmark of this phenotype\textsuperscript{4,5}. In addition, from 2001, the deficiency of collagen VI in muscle has been associated with Ullrich scleroatonic congenital muscular dystrophy (UCMD) that is caused by different types of recessive and dominantly acting mutations in the same three collagen VI genes\textsuperscript{4,6-8}. UCMD is clinically less heterogeneous than Bethlem myopathy; however, although the majority of patients have the classic severe form that is characterized by neonatal muscle weakness, proximal joint contractures, hyperlaxity of the distal joints and severe course\textsuperscript{4}, milder patients have now been reported\textsuperscript{9}.

Both, Ullrich and Bethlem phenotypes are linked to the COL6A1, COL6A2 or COL6A3 genes, encoding respectively the alpha 1, alpha 2 and alpha 3 chains of collagen VI, and show clinical and genetic heterogeneity; therefore mutation detection in essential in these disorders for allowing the correct diagnosis, the establishment of prognosis and an accurate genetic counseling.

For emphasizing this clinical and genetic heterogeneity, we report on two patients with distal joint hyperlaxity, the first with a mild to moderate myopathic phenotype including joint hyperlaxity and the second with a severe Ullrich phenotype. In both, a molecular analysis was performed at the Thomas Jefferson University, Philadelphia, by Pan et al.\textsuperscript{7} and revealed in the first a dominantly acting mutation in the COL6A1 gene, that has not been described yet, and in the second a heterozygous in-frame deletion in the COL6A1 that has been previously described in Bethlem myopathy\textsuperscript{10,11}.

**METHOD**

Sixty children with clinical and histopathological diagnosis of congenital muscular dystrophy (CMD) had their muscle samples evaluated immunohistochemically by means of immunofluorescency or immunoperoxidase methods, utilizing antibodies for dystrophin (C-terminal), merocyan (80 Kda and 300 Kda), sarcoglycans (\(\gamma, \gamma', \gamma\) and \(\gamma\)-SGs) and dystroglicans (\(\gamma\)-DG and \(\gamma\)-DG)\textsuperscript{12}. Among them, 7 presented marked distal hyperlaxity and had their muscle samples also tested for collagen VI immunoreactivity using Hybridoma Bank antibody, code 5C6, 1/100. In two patients (Cases 1 and 2) collagen VI immunoreactivity was absent.

**CASES**

Case 1 – A 4 year-9 month-old male was born at term following an uneventful pregnancy from non consanguineous parents who had already two healthy children. At birth, the boy presented bilateral hip dislocation that was treated by the pediatrician and orthopedist. Motor development was mildly delayed: the child acquired supported walking by 15 months of age and unsupported walking by 21 months of age. From the age of two years, frequent falls and a difficulty for running and climbing stairs were noted by the parents and other relatives. Language and mental development were normal. Our first examination at 4 years of age revealed, a mild to moderate difficulty for getting up from the floor, a mild proximal weakness of the four limbs (MRC 4), a marked generalized hypotonia, as well as a striking and widespread joint hyperextensibility. Deep tendon reflexes were hypoactive. On physical examination lumbar lordosis and a few small areas of abnormal hypochromic pigmentation in the skin of the lower limbs were noted. Serum creatine kinase levels were two-fold increased and electromyography denoted abnormal myopathic pattern of muscle discharges. A muscle biopsy was performed at 5 years and 2 months of age and revealed mild to moderate dystrophic changes represented by size fiber variability, moderate perimysial fibrous infiltration, mild endomysial fibrous infiltration, a scarce fatty deposition and some necrotic fibers. Cardiac evaluation was normal. After a follow-up of 39 months, the course can be considered slowly progressive as we observed a worsening of proximal muscle weakness (MRC 3 to 4) and the installation of mild distal weakness (MRC 4). The joint hyperlaxity persists and the boy did not develop any joint contracture. A molecular study of the patient’s DNA was done at the Department of Dermatology and Cutaneous Biology, Jefferson Institute of Molecular Medicine, Thomas Jefferson University, Philadelphia, by Pan et al.\textsuperscript{7} and demonstrated a Bethlem myopathy heterozygous in-frame deletion in the COL6A1 gene, that had been previously described\textsuperscript{10,11}. The patient’s father has normal posture, mus-

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LM, laminin; col, collagen; dys, dystrophin; SG, sarcoglycan; DG, dystroglycan; P, positive immunoreactivity, absent immunoreactivity.
cular strength and tendon reflexes but presents moderate hyperlaxity of both thumbs, the left hand fingers and the left elbow. The analysis of his DNA did not reveal any mutation.

Case 2 – A 6-year-old boy was born at term following an uneventful pregnancy from non consanguineous parents who had already two healthy children. The child presented from birth severe congenital hypotonia and generalized muscle weakness with proximal predominance. Motor development was delayed: he sits without support by the age of one year and never acquired independent walking. Language and mental development were normal. From the second year of life he gradually developed elbows and knees contractures. Our first examination at 6 years of age revealed decreased muscular strength [score of 3 and 2, following the Medical Resource Council (MRC) scale, respectively in the distal and proximal segments of the 4 limbs] widespread muscular hypotonia and hypotrophy, distal joints hyperlaxity, as well as absent deep tendon reflexes. We noted a mild ankles protrusion which the parents referred as more pronounced in the past years. The mother considered, after being inquired, that the child has hyperhidrosis when compared to his two normal older siblings. Cardiac evaluation was normal. Serum creatine kinase levels were normal and electromyography revealed abnormal myopathic pattern of muscle discharges. The first muscle biopsy, performed at the age of 6 years, showed moderate size fiber variability, and mild to moderate perimysial as well as endomysial fibrous infiltration. A second biopsy was performed at 9 years of age and evidenced marked worsening of the former aspects and an additional accentuated fatty deposition, as well as some necrotic fibers. Immunohistochemical analysis with different antibodies was done (Table 1) and showed no collagen VI immunoreactivity (Fig 1). In a previous study, the patient’s muscle sample had also been analysed for laminins 1, 1, 2 and 1 chains immunoreactivity. The result was normal, i.e. negative immunomarcation for 1 laminin chain, striking immunomarcation for 1 and 1 laminin chains and a little less pronounced immunomarcation for 2 laminin chain. Along the 8 years of follow-up, the boy manifested a progressive worsening, that was characterized by an accentuation of the hipotrophy and of the contractures which became widespread, as well as by the installation of scoliosis. Currently, the boy is 14-year-old and depends on ventilatory support from 11 years of age. A molecular analysis was performed at the Department of Dermatology and Cutaneous Biology, Jefferson Institute of Molecular Medicine, Thomas Jefferson University, Philadelphia, by Pan et al. and revealed a de novo in-frame heterozygous deletion of the COL6A1 gene.

DISCUSSION

Collagen VI is a protein that provides a microfibrillar network in the extracellular matrix of the muscular tissue, as well as in other organs. It is essential for the correct function of muscle fibers, maintaining its structural integrity. An animal model of human Bethlem myopathy was already described and the details about the composition and the role of collagen VI have been widely discussed.3

Bethlem myopathy and Ullrich CMD result from molecular changes in each one of the three genes encoding collagen VI. The exact mechanism by which collagen VI leads to the myopathy is not

![Fig 1. Immunohistochemical analysis of collagen VI in muscles samples, utilizing Hybridoma Bank antibody, code 5C6,1/100. On the left: normal control. On the right: muscular biopsy of patient 2.](image-url)
perfectly clear. Recently, a possible mitochondrial dysfunction in myofibers has been implicated in this mechanism. According to Mercuri et al., collagen VI involvement is associated to molecular changes in collagen VI genes in near to 40% of the CMD patients with Ullrich phenotype. In addition, there are reports of patients with low collagen VI reactivity without mutations in collagen VI genes, as well as of patients with mutations in collagen VI genes without changes in collagen VI reactivity, therefore documenting the genetic heterogeneity of Ullrich phenotype. Although the role of collagen VI seems to be excluded in a number of cases, Ishikawa et al. recently considered that in patients with Ullrich phenotype who have no mutations in the collagen VI genes and therefore a normal amount of Collagen VI in the interstitium, a primary abnormality of other not yet identified molecules could cause a failure of collagen VI to anchor the basal lamina to the interstitium.

The first description of Bethlem myopathy was referred by Bethlem & van Wijngaarden, who in 1976 reported 28 patients from three families with an autosomal dominant, benign and slowly progressive myopathy. The most characteristic aspect of Bethlem myopathy is the occurrence of early contractures of the interphalangeal joints and the elbows. Merlini et al. considered that the fingers contractures are the hallmark of Bethlem myopathy. Clinical presentation and age of onset are highly variable and although the clinical course of the disease is thought to be benign, some reports emphasize that Bethlem myopathy can be slowly progressive and can culminate in wheelchair use. Histopathological findings were either nonspecific or compatible with dystrophic changes and creatine kinase levels can be normal or mildly elevated. Collagen VI can be normal. As different kinds of mutations have been found in Bethlem myopathy, there are attempts of establishing genotype/phenotype correlation in Bethlem patients and some data indicate that large deletions and mutations inside the triple-helical collagen VI monomer helix formed by the three collagenous polypeptides γ1, γ2 and γ3 are associated with a more severe phenotype than those occurring in the N-terminal globular region.

The first report of Ullrich phenotype occurred in 1930 by Ullrich who named it scleroatonic form of CMD and until 2002 only recessive mutations had been described in patients with UCMD. In 2003, the first dominantly acting mutation in the COL6A1 gene was found in one of our Brazilian patients who are now reporting and recently more three patients with a dominantly acting mutation in the COL6A1 gene were published. Besides the genetic heterogeneity, UCMD also exhibits clinical heterogeneity that is not related to each of the 3 loci, but can be associated to the degree of the deficiency of collagen VI in muscle or cultured fibroblasts. A complete deficiency has been observed in the severe cases while the milder ones show a partial deficiency. However, the majority of patients have a severe involvement that includes scoliosis, failure to thrive, and early and severe respiratory impairment by the end of the first decade of life. Mildly affected patients can be related to mutations leading to a partial deficiency of collagen VI.

The present report intends to emphasize the wide spectrum of phenotypes that can be associated to collagen VI deficiency. Both patients have marked distal hyperlaxity, and histopathological dystrophic pattern, but clinical involvement was essentially different: the first (with 3 years and 4 months of follow-up) acquired independent walking and shows a mild difficulty for running and climbing; the second (with 8 years of follow-up) never acquired independent walking and needs intermittent ventilatory support from the beginning of the second decade of life. A molecular study of both patients was performed by Pan et al. at the Thomas Jefferson University and demonstrated in each one a different type of deletion of COL6A1 gene: in the first a heterozygous in-frame deletion in the COL6A1 that has been previously described in Bethlem myopathy and from 2003 is associated also to UCMD, as well as with a particular aspect of ossification of the posterior longitudinal ligament of spine in some subjects.

In Patient 1, the absence of contractures, the marked joint hyperlaxity, and the dystrophic pattern found on muscle biopsy had been supposed by us as suggestive of a non specific merosin-positive CMD diagnosis. The result of the molecular analysis denoting a previously described Bethlem myopathy heterozygous in-frame deletion in the COL6A1 gene indicates that the boy, currently young, will probably manifest contractures and develop along the follow-up a phenotype more compatible...
ble with Bethlem myopathy. However, as his follow-up is now completing three years, he can be considered an atypical case. During the 100th European Neuromuscular Center (ENMC) international workshop, Muntoni considered that joint laxity, affecting especially the knees and elbows, can be a common finding at presentation and disappears along the years. In the same opportunity, this author reported a case particularly coincidental to ours including by the presence of bilateral hip dislocation. His patient, currently aged 28, has also congenital torticollis, a finding that has been commonly described. In addition, the dystrophic changes on the muscular biopsy, previously considered non compatible with Bethlem myopathy, have been found so frequently as the non specific changes. Mercuery et al. reported that the degree of endomysial connective tissue is rarely observed. Jobsis et al. followed-up 23 children and 36 adult patients with Bethlem myopathy and found that nearly all children exhibit weakness or contractures during the first two years of life. In addition, according to Bertini and Pepe, muscle biopsy from Bethlem cases shows non specific changes and an increase of endomysial connective tissue is rarely observed. Mercuery et al. reported that the degree of muscle involvement varies according to the degree of motor impairment. Therefore, although Muntoni and Voit have referred that UCMD is probably the second most frequent variant of CMD, it is our impression that UCMD is not so common among Brazilian patients.

In conclusion, the new molecular data seem suggest that new phenotypes linked to collagen VI unit and particularly to COL6A1 gene can be identified in a next future, so defining if Ullrich and Bethlem phenotypes are independent entities or, as reported by Bertini and Pepe, represent an overlap between the clinical phenotypes and the molecular defects. A probable overlap between UCMD, Bethlem myopathy and Ehlers-Danlos syndromes has been the focus of recent researches. The search for new mutations in the three genes of collagen VI unit in all patients with typical Ullrich phenotype, typical Bethlem phenotype and non specific merosin-positive CMD phenotype associated to joint hyperlaxity, as well as the description of each phenotype associated to the new mutations represent an enormous field of researches in infantile myology, particularly for clarifying undefined merosin-positive CMD forms. In addition, new molecular and clinical descriptions are needed for reaching a better understanding of the role of collagen VI in the muscle function and its correlation with the other collagen units.


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REFERENCES