

Research Report

Laminin $\alpha 2$ Deficiency-Related Muscular Dystrophy Mimicking Emery-Dreifuss and Collagen VI related Diseases

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Abstract.

Background: Laminin $\alpha 2$ deficient congenital muscular dystrophy, caused by mutations in the *LAMA2* gene, is characterized by early muscle weakness associated with abnormal white matter signal on cerebral MRI.

Objective: To report on 4 patients with *LAMA2* gene mutations whose original clinical features complicated the diagnosis strategy.

Methods: Clinical, electrophysiological, muscle imaging and histopathological data were retrospectively collected from all patients. DNA samples were analysed by next-generation sequencing or direct gene sequencing. Laminin $\alpha 2$ was analysed by western-blot and immunohistochemistry.

Results: The four patients achieved independent walking. All had proximal muscle weakness with scapular winging and prominent joint contractures without peripheral neuropathy. During follow-up, two patients suffered from refractory epilepsy associated with brain leukoencephalopathy in one, polymicrogyria and lissencephaly without white matter changes in the other. In two patients, the distribution of fatty infiltration resembles that of collagen-VI related myopathies. Dilated cardiomyopathy

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with conduction defects, suggestive of Emery-Dreifuss myopathy, emerged in two of them within the 4th decade. Molecular diagnosis remained elusive for many years. Finally, targeted capture-DNA sequencing unveiled the involvement of the *LAMA2* gene in two families, and led us to further identify *LAMA2* mutations in the remaining family using Sanger sequencing.

Conclusions: This report extends the clinical and radiological features of partial Laminin $\alpha 2$ deficiency since patients showed atypical manifestations including dilated cardiomyopathy with conduction defects in 2, epilepsy in 2, one of whom also had sole cortical brain abnormalities. Importantly, clinical findings and muscle imaging initially pointed to collagen-VI related disorders and Emery-Dreifuss muscular dystrophy.

Keywords: Laminin alpha 2, *LAMA2*, congenital muscular dystrophy, Emery-Dreifuss muscular dystrophy, collagen VI-related myopathy, dilated cardiomyopathy

INTRODUCTION

Congenital muscular dystrophy (CMD) is a clinically and genetically heterogeneous group of neuromuscular disorders [1]. Laminin alpha 2-deficient CMD (MDC1A, OMIM #607855) is characterized by hypotonia at birth or within the first few months of life, contractures of large joints, elevated serum creatine kinase (CK) levels and abnormal white matter changes although cognitive abilities are normal [2–4]. MDC1A, caused by a deficiency in the $\alpha 2$ chain of laminin-211 [5] previously named merosin [6], is the most represented form of CMD [7]. Typically, independent ambulation is not achieved in MDC1A with complete laminin-alpha 2 deficiency. Only a few reports have mentioned cardiac involvement associated with MDC1A (for reviews see [8, 9]). Atypical presentations such as adult onset limb-girdle muscular dystrophies with white matter abnormalities have been reported with partial laminin-alpha 2 deficiency [10, 11]. Numerous mutations of the *LAMA2* gene encoding the $\alpha 2$ chain of laminin-211 have been identified, scattered along the 64 coding exons and leading to partial or complete protein deficiency [4].

Here we report four adult patients in whom the initial diagnosis suggested by clinical presentation and muscle biopsy findings was mainly consistent with either Bethlem myopathy (BM) or Emery-Dreifuss muscular dystrophy (EDMD). Peculiar features included dilated cardiomyopathy (two patients) and epilepsy (two patients) that occurred during follow-up. Ultimately, molecular diagnosis was achieved through next generation sequencing (NGS), revealing homozygous or compound heterozygous *LAMA2* gene mutations. These findings extend the clinical spectrum of *LAMA2* mutations to adults, clinically resembling Bethlem myopathy or Emery-Dreifuss muscular dystrophy and emphasize further the critical input of NGS in diagnosing atypical forms of myopathies.

MATERIAL AND METHODS

Clinical assessment

Four patients including two isolated cases (Patients #1 and #2) and two brothers (Patients #3 and #4) were included in this study (Fig. 1). Neurological assessment, CK levels, muscle imaging including whole-body muscle MRI for patients #1 and #2 and muscle CT-scan for patients #3 and #4, cardiac investigations including at least electrocardiogram (ECG) and echocardiography, pulmonary function tests, electroneuromyography and brain MRI or CT scan were retrospectively collected from medical files for each patient (Table 1). All biological samples (blood, skin and muscle biopsies) were obtained after informed consent from patients and their relatives.

Histopathology, immunohistochemistry

Muscle biopsy was performed in 3 out of the 4 patients. Biopsy specimens of deltoid muscle were analysed by light microscopy [12]. Immunohistochemical studies were carried out for all patients on 10 μm cryosections for dystrophin, α -, β - and γ -sarcoglycans, dysferlin, α -dystroglycan, desmin, αB -crystallin, myotilin, myosin heavy chain, caveolin 3 and collagen VI. Laminin $\alpha 2$ chain immunostainings were performed after *LAMA2* mutations identification and used 3 antibodies directed against the whole protein (NCL-merosin, Leica Novocastra), the N-terminal 300 KDa fragment (4H8-2, Abcam) or the C-terminal 80KDa fragment (MAB 1922, Chemicon International). Representative images were obtained with an Axioplan 2 microscope (Zeiss) equipped with a HBO 100 mercury lamp (Zeiss) and captured using Metaview software (Roper Scientific), using identical exposure time for each antibody.

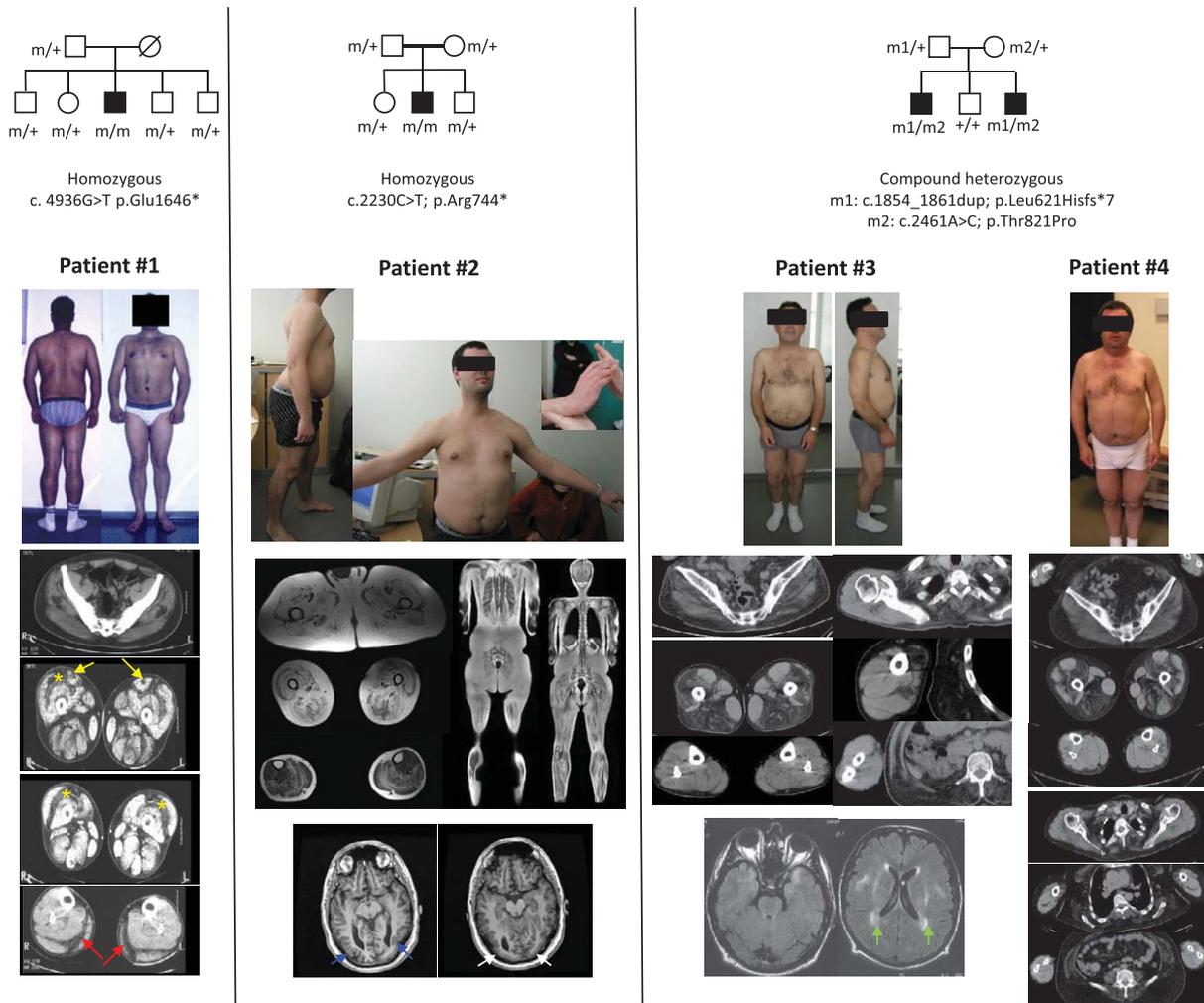


Fig. 1. Patients carrying *LAMA2* mutations. Patient #1 presents distal and proximal joint contractures. The muscle CT scan showed a predominantly central hypodensity in the rectus femoris (yellow arrows), in the peripheral region of the *vastus lateralis* (asterisks) and the gastrocnemius medialis and lateralis muscles (red arrows). Patient #2 also presents distal and proximal joint contractures. Whole body MR showed diffuse fatty infiltration of pelvic. The superficial layers of the *vastus lateralis*, *semimembranosus*, *semitendinosus* and *gracilis* muscles were less affected. The fatty infiltration surrounded the *soleus* muscle and was localized at the periphery of *gastrocnemius medialis* and *lateralis*, whereas the *tibialis anterior*, the extensors and the *tibialis posterior* muscle were almost spared. Brain MRI disclosed a posterior polymicrogyria (blue arrows) and lissencephaly (white arrows). Patient #3 and #4 have mild ankle and elbow contractures. Muscle CT scan showed marked hypodensity of the *vastus lateralis* and *intermedius*, the *semimembranosus*, the *semitendinosus*, the *biceps femoris* and the adductor muscles, paraspinal muscles, mild involvement of right *peroneus longus* muscle in patient #3 and *peroneus longus* and *extensor digitorum longus* muscles in patient #4, whereas the *gracilis*, *sartorius*, *vastus medialis*, *rectus femoris*, *gastrocnemii*, *soleus*, *tibialis anterior* muscles were spared. In the upper limbs, deltoid and biceps brachialis muscles present a mild fatty infiltration. Brain MRI of patient #3 showed a symmetric leukoencephalopathy (green arrows).

Western blot analyses

Western blot analyses were performed on proteins extracted from muscle biopsy for dystrophin, α -, β - and γ -sarcoglycans, calpain, dysferlin, α -dystroglycan in all patients, and from lymphoblastoid cell lines (LCL) for emerin in patient #3 according to previous

studies [13, 14]. After *LAMA2* mutations identification, laminin $\alpha 2$ western blot was performed on muscle samples from patients #1, #2 and #3 as well as in 2 control subjects and a previously identified MDC1A patient with complete laminin $\alpha 2$ deficiency using the C-terminal 80KDa fragment antibody (MAB1922, Chemicon International).

Collagen VI immunolabelling on cultured fibroblasts

Skin biopsies were obtained from patients #1 and #2 and confluent fibroblasts cultures were subjected to two different protocols to detect collagen VI expression and secretion as previously described [15].

Genetics

DNA samples were extracted from blood using standard methods. Linkage studies were conducted using the Illumina Linkage-24 beads array platform, covering around 6000 SNPs and analysed using the Merlin software [16]. As one of the partners of the EU FP7-NMD-Chip consortium, we used the DNA capture array designed in this European project [17]. For patients #1 and #3 who developed cardiomyopathy during the course of their disease, 46 genes involved in cardiomyopathies were studied through a targeted DNA capture assay (TruSight Cardiomyopathy, Illumina). Validation and segregation of variants were subsequently performed using the Sanger sequencing method.

RESULTS

Clinical findings

The main clinical and muscle imaging findings in the 4 patients are reported in Table 1 and Fig. 1.

Patient #1 is of Turkish origin with no known parental consanguinity. He was first seen before he developed any cardiac disease. He was clinically diagnosed with Bethlem myopathy at 28 years of age, mainly due to keloid scars and joint contractures including Achilles tendons (lengthened at 15 and 25 years old) and finger flexors requiring surgical repair at 25. At 25 years of age, the muscle CT scan pattern was compatible with a collagen-VI related myopathy. Indeed, the *rectus femoris* had an area of central hypodensity. The fatty infiltration was localized in the peripheral region of the *vastus lateralis*. The *semimembranosus*, *semitendinosus* and *biceps femoris*, as well as *adductor* muscles were only mildly involved, whereas the *gracilis* was spared. In the legs, the fatty infiltration was localized between the *gastrocnemius (lateralis and medialis)* and the *soleus*. He then progressively developed dilated cardiomyopathy from his 30's that suggested a diagnosis of EDMD.

Patient #2 is the eldest boy of consanguineous parents from Algeria. He achieved independent ambulation at 13 months, despite severe hypotonia during his first months of life. For a long time he was considered as suffering from an autosomal recessive form of limb girdle muscular dystrophy (LGMD). He experienced a femur fracture at 16 years which led to a worsening of lower limb muscle weakness. He was wheelchair dependent at 27 years although he could walk 5 meters at his last assessment at 32 years old. Muscle MRI, performed at 29 years of age, showed severe and diffuse fatty infiltration of the proximal muscles, lumbar extensors, and abdominal wall muscles. By contrast, the superficial layers of the *vastus lateralis*, the *semimembranosus*, the *semitendinosus* and the *gracilis* muscles were less affected. In the legs, the fatty infiltration surrounded the *soleus* muscle and was localized at the periphery of *gastrocnemius medialis* and *lateralis*, whereas the *tibialis anterior*, *extensor* and *tibialis posterior* muscles were almost preserved. Atypical features in the LGMD context included mixed partial and generalized epilepsy that he developed from 6 years of age which was successfully treated with carbamazepine until his 20s. Afterwards, he developed severe pharmacoresistant partial-onset epilepsy with up to 30 partial seizures per day. Various antiepileptic drugs were tried that did not significantly change seizures frequency, except for Vigabatrin which was finally withdrawn because of visual field defects. Extensive brain imaging revealed bilateral occipital lissencephaly and polymicrogyria on T1 weighted sequence, without any white matter changes. Cardiac assessment was always normal during his follow-up. Occurrence of extensive joint contractures, follicular hyperkeratosis without keloids pointed to collagen VI-related myopathy as a possible diagnosis.

Patients #3 and #4 are brothers from non-consanguineous Portuguese parents. They had normal motor development and are still ambulatory at 50 and 54 years respectively. Due to extensive joint contractures, the presence of premature ventricular contractions and left ventricular dysfunction found in the youngest brother since 37 years of age, EDMD was suggested. Muscle CT scans, performed at 43 and 47 years of age, respectively, showed at the thigh level advanced hypodensity of the *vastus lateralis* and *intermedius* muscles, the *semimembranosus*, the *semitendinosus*, the *biceps femoris* and the adductors whereas the *gracilis*, *sartorius*, *vastus medialis* and *rectus femoris* muscles were spared. At the leg level, *peroneus longus* and *extensor digitorum longus*

Table 1
Summary of the clinical features of the 4 patients

Case #	Age at last assessment	First symptoms / age at onset	Maximal motor achievement / Walkon scale at last assessment	CK level (X / normal level)	Muscle weakness (X / normal level)	Joint Contractures	Fatty involvements on muscle imaging	Epilepsy (age at onset) / Treatment	Brain imaging	Cardiac involvement / Last LVEF (%) / FVC (%)	Other features	LAMA2 mutation (Status, acid changes)
1	38	Calves myalgia at running, tip-toe walking / 10y	Wt at 1 / 6	8X	Prox. (LL+UL), mild scapular winging	N, A, E, W, FF	GMa, GMe, GMm, all anterior and posterior compartment thigh muscles, deeper layers of GM, GL, VL, RF (central hypodensity)	No	Normal	DCM, sinus rhythm, VT, ICD at 35 y / 30	Calf hypertrophy	Homozygous, c.4936G>T, p.Glu1646*
2	32	Severe hypotonia but achieved walking at 1 y / birth	Wt at 1 / 3	2.5X	Prox. (UL+LL), axial (flexors, extensors), scapular winging	N, A, H, E, W, FF	Periphery of LL, UL and axial muscles	Partial epilepsy (6 y) / Carbamazepine until 20 y then pharmacoresistant	Hippocampal polymicrogyria, complex bilateral cortical dysplasia	No / normal	Follicular hyperkeratosis	Homozygous, c.2230C>T, p.Arg744*
3	49	Spine and elbows stiffness / 6y	Wt at 1 / 3	4X	Prox. (LL+UL), scapular winging	Sh, E, W, FF, K, A, RS	Del, BB, BH, GMa, GMe, GMm, PS, VL and hamstrings, and paraspinal muscles, right PL	No	Brain MRI = Multiple and bilateral white matter hypersignals within frontal, parietal and temporal regions	DCM, sinus rhythm, resuscitated sudden death due to VF / 35	Quadriceps and calf hypertrophy	Compound heterozygous, c.1854, 1861dup; p.Leu621Hisfs*7 / c.2461A>C, p.Thr821Pro
4	47	Delayed walking and frequent falls / 2.5y	Wt at 2.5 / 3	3X	Prox. (LL+UL), no scapular winging	Sh, E, FF, K, A, RS	Del, BB, BH, GMa, GMe, GMm, PS, VL, VI, all hamstrings muscles asymmetrically affected, paraspinal muscles, PL and EDL, sparing of VM, RF, SA, AL	Post-traumatic generalized epilepsy (18y) successfully treated by phenobarbital, sodium valproate and finally by carbamazepine and diazepam	Brain MRI = Multiple and bilateral white matter hypersignals in a patchy pattern within periventricular and infracortical regions involving also temporal lobes	No / 58	VL hypertrophy	Compound heterozygous c.1854, 1861dup; p.Leu621Hisfs*7 / c.2461A>C, p.Thr821Pro

A: ankles, AL: adductor longus muscle, BH: biceps humeri muscle, CK: creatine kinase; DCM: dilated cardiomyopathy; E: elbows, ENMG: electromyography; FF: finger flexors, FVC: forced vital capacity; GM : gastrocnemius medial head, GMa, GMe, GMm: gluteus maximus, medius and minimus muscles, GL: gastrocnemius lateral head, GR: gracilis muscle, H: hips, ICD: implantable cardioverter defibrillator; K: knees, LL: lower limbs; MP: myopathic pattern; MRI: magnetic resonance imaging; N: neck, NCV: nerve conduction velocities, PM: pacemaker; Prox (proximal), PS: psoas muscle, RF: rectus femoris muscle, RS: rigid spine, SA: sartorius muscle, Sh: shoulders, UL: upper limbs, VA: ventricular arrhythmia, VM: vastus medialis muscle, VI: vastus intermedius muscle, VL: vastus lateralis muscle, W: wrists, WI: walked independently, Y: year.

muscles were mildly affected in patient #4 while only the right *peroneus longus* muscle was mildly affected in patient #3. The other leg muscles including *tibialis anterior*, *soleus*, *gastrocnemii* and *tibialis posterior* were spared. In the upper limbs, *deltoid* and *biceps brachialis* muscles had a mild fatty infiltration. Atypical features included *triceps brachii*, *quadriceps* and *gastrocnemius* muscles hypertrophy and abnormal brain MRI that showed leukoencephalopathy. In addition, patient #4 developed several episodes of loss of consciousness with eye rolling and postictal amnesia, unresponsiveness preceded by blurred vision, paraesthesia in the lower limbs and spreading to the head. EEG was either normal or showed bi-occipital spike-waves predominating within the right side. Extensive neurological investigations including cerebrospinal fluid analysis, sensory and auditory evoked responses were normal while visual evoked responses showed delayed central and peripheral responses. Ophthalmologic examination, skin and rectal biopsies, arylsulfatase, beta-galactosidase, hexosaminidase and long chain fatty acid dosage were normal. The epilepsy was assumed to be post-traumatic and the patient was treated by phenobarbital, sodium valproate and finally by carbamazepine and diazepam.

CK levels were usually elevated in all patients ranging from 2.5 to 8 times normal levels. All electroneuromyographic studies, performed in adulthood, showed normal nerve conduction studies and myogenic pattern at needle electromyography.

Histological, immunohistological and biochemical findings

Muscle biopsies for patients #1 and #2 showed nonspecific dystrophic features including increased internal nuclei and connective tissue, leading to an initial suspicion of BM (Fig. 2A). Muscle biopsy from patient #3 revealed fibre size variation, moderate endomysial fibrosis, atrophic angulated myofibers and fiber type grouping suggesting a neuropathic pattern (data not shown). Routine immunohistochemical stainings, including α -dystroglycan, showed normal expression of all proteins analysed in the patients (data not shown). Muscle biopsy multiplex western blotting demonstrated that dystrophin, α - and γ -sarcoglycan, calpain 3, dysferlin and α -dystroglycan, were normally expressed, except in protein extracts from patient #3, which revealed mildly reduced amounts of α - and γ -sarcoglycan (data not shown). Western blot analysis on lymphoblastoid cells from patient #3 revealed normal emerin expression (data not shown). Collagen VI

immunolabelling was normal in muscle cryosections and cultured dermal fibroblasts from patient #1 while reduced COLVI secretion was detected in fibroblasts from patient #2 (data not shown).

Genetic studies

According to the diagnostic pathway tree for each patient, several genes including *LMNA* (patients #1–3), *EMD* (patients #1–3), the 3 collagen VI encoding genes *COL6A1-3* (patients #1 and #2), *SGCA* and *SGCG* (patient #3), *FKRP* (patients #1 and #3), *FHL1* (patients #1 and #3), *CAPN3* and *DES* (patient #1) and *FSHD-1* (patient #2), were screened and ruled out. Importantly, no pathogenic mutation was found in patients #1 and #3 in the panel of 46 genes known to be associated with isolated or syndromic cardiomyopathies with or without skeletal muscle involvement. In parallel, linkage analysis was performed on patient #2 family and two regions presented a lod-score >1.8 (on chr2 and chr6).

In the context of the European NMD-Chip project, we turned to NGS technologies under the hypothesis of a recessive mode of inheritance. We identified a novel nonsense *LAMA2* (NM.000426.3) mutation in patient #1 (c.4936G>T; p.Glu1646*) and a previously reported one in patient #2 (c.2230C>T; p.Arg744*) [18]. Both mutations are homozygous in the patients and lead to premature termination codons (PTC) in the laminin α 2 chain (Table 1, Fig. 1). Mutations were confirmed by Sanger sequencing and screened in other family members. The father and the four non-affected siblings of patient #1 carried the c.4936G>T mutation in a heterozygous state, while the parents and the two non-affected siblings of patient #2 carried the c.2230C>T mutation in a heterozygous state. In light of these *LAMA2* mutations and of the phenotypic similarities between patients #1 and #2 and the 2 brothers from the remaining family (patients #3 and #4), the 65 *LAMA2* exons were directly sequenced in the latter with the Sanger method. Two compound heterozygous variants were identified (Table 1, Fig. 1): the first (c.1854_1861dup; p.Leu621Hisfs*7) had already been described as pathogenic [19] and the second (c.2461A>C; p.Thr821Pro) was previously submitted to the Leiden muscular dystrophy pages *LAMA2* database (www.dmd.nl/). It has been predicted pathogenic by SIFT, Polyphen2 and UMD predictor tools [20]. The c.2461A>C was found at heterozygous state in the mother, whereas the c.1854_1861dup was transmitted by the father. The unaffected brother of the patients did not carry any of these mutations (Fig. 1).

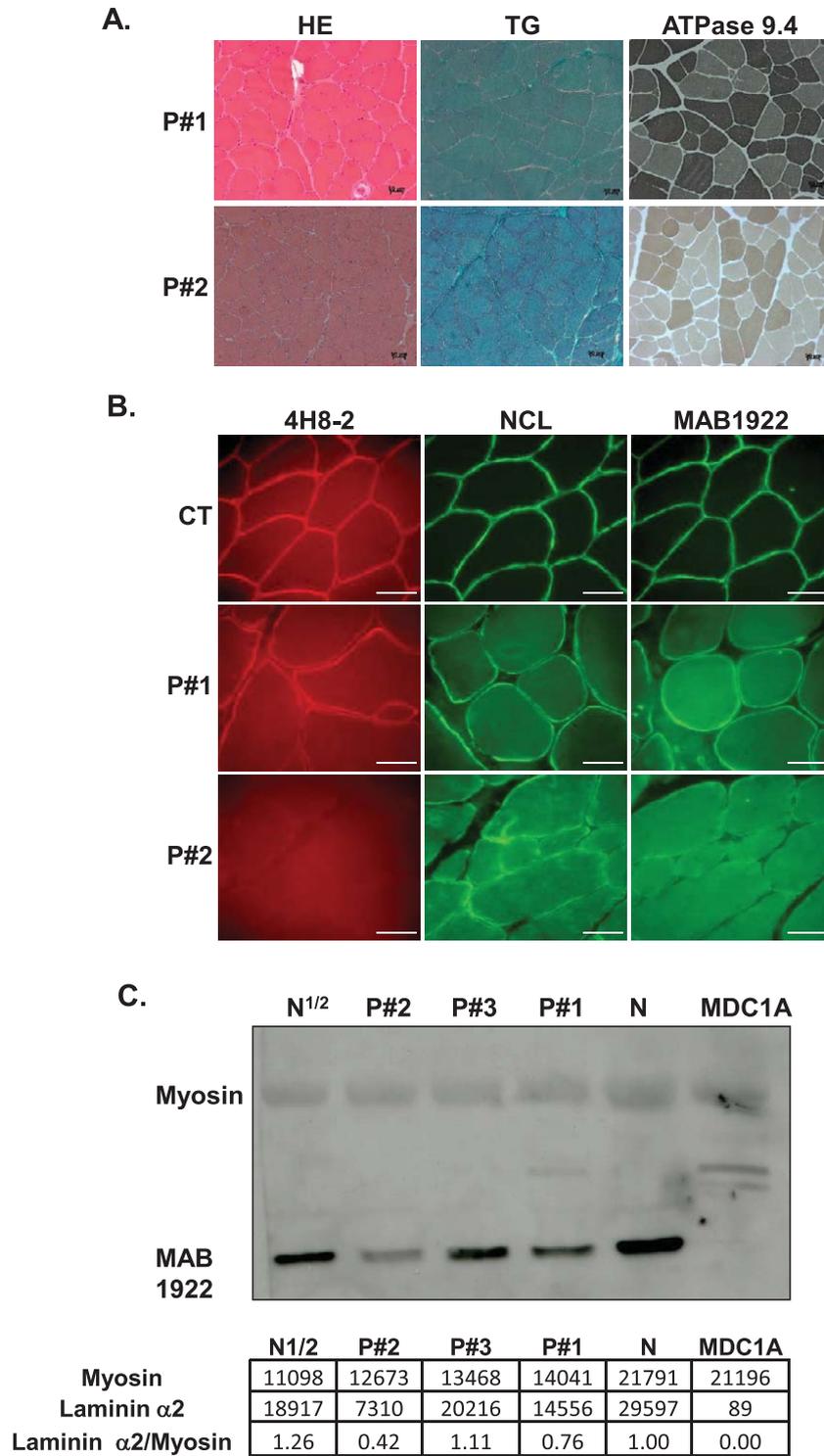


Fig. 2. Muscle histology, immunolabelling with laminin α 2 antibodies and western blot studies. Histology of muscle biopsies from patients #1 and #2 using Haematoxylin Eosin, modified Gomori trichrome and ATPase pH 9.4 are presented. Bar: 50 μ m. (B) Immunostaining on muscle cryosections from a control individual (CT), patient #1 and patient #2. Three antibodies against different regions of the α 2 chain of laminin were used: 4H8-2, NCL and MAB1922, Bar: 50 μ m. (C) Western blot analysis of muscle biopsies from patients #1–3, using MAB1922 antibody with densitometry values below the blot.

Laminin $\alpha 2$ expression analyses

Immunohistochemical stainings of laminin $\alpha 2$ chain in patient #3, performed prior to any genetic analyses, was considered as normal (data not shown). In patients #1 and #2, laminin $\alpha 2$ chain stainings were performed post-mutation identification and were found to be abnormal (Fig. 2B), associated with an increased expression of laminin $\alpha 5$ (data not shown). In fact, patient #1 showed only slight irregularities and weakness of laminin $\alpha 2$ staining while patient #2 showed absence of laminin $\alpha 2$ staining when using the N-terminal 300 KDa and the C-terminal 80KDa fragment antibodies and a moderate reduction with the whole protein antibody (Fig. 2B). All these data were consistent with laminin $\alpha 2$ partial deficiency. Interestingly, western-blot studies in the 3 patients showed normal size but reduced laminin $\alpha 2$ chain amounts in muscle biopsy extracts particularly in patients #1 and #2 (Fig. 2C). For patient #3, laminin $\alpha 2$ amount appears to be reduced by half (Fig. 2C).

DISCUSSION

The clinical presentation of most patients carrying *LAMA2* gene mutation is relatively similar with severe congenital hypotonia, delayed milestones with inability to achieve independent ambulation, extensive joint contractures, muscle weakness, normal mental development associated with white matter abnormalities revealed by brain MRI, and markedly raised CK levels [21, 22]. However, milder forms presenting as LGMD have been reported [10, 23]. Additional features have been rarely described [24] and may include subclinical cardiac involvement and neuronal migration defects associated with diffuse white matter changes leading to variable degrees of epilepsy and mental retardation [11, 23, 25, 26].

From the clinical point of view, our patients came to our attention at an adult age for diagnosis purpose after a long atypical disease course and the molecular diagnosis proved challenging. Over the years, the diagnostic hypothesis varied from BM (Patients #1 and #2) to EDMD (Patients #1, #3 and #4) and even to LGMD (Patients #2 and #4). However no mutation was found in the main candidate genes. The presence of extensive joint contractures combined with muscle imaging pattern resembling collagen VI related myopathy (Patient #1), follicular hyperkeratosis with non-conclusive muscle MRI pattern (Patient #2) led to misdiagnosis in these 2 patients. Although

collagen VI secretion was reduced in cultured fibroblasts, *COL6A1-3* genes screening remained normal in patients #1 and #2. We suspect this deficit in COLVI secretion to be non-specific and likely secondary to an as of yet uncharacterized altered homeostasis of the dermal fibroblasts from the patient. To our knowledge this pattern of fatty infiltration surrounding the muscle has not been yet reported in *LAMA2* mutated patients. Therefore, *LAMA2* gene analysis is warranted in patients with prominent joint contractures, especially those with secondary collagen VI abnormalities [27]. Patient #2 also presented with pharmacoresistant epilepsy and extensive bilateral occipital micropolygyria. Epilepsy and focal cortical dysplasia associated with cognitive deterioration have been reported in addition to the classical diffuse white matter abnormalities in some cases with total laminin $\alpha 2$ deficiency [25, 28, 29] and more recently in a 7 year-old patient with epilepsy, cognitive deterioration, extensive bilateral occipital polymicrogyria who lost ambulation [30]. These reports are in contrast with patient #2 who displayed only partial laminin $\alpha 2$ deficiency and is still able to walk a few steps within his 4th decade of life. Indeed patients showing a LGMD-like form of laminin $\alpha 2$ -deficient CMD were already pointed out previously, indicating that partial laminin $\alpha 2$ deficiency should be kept in mind in the work-up diagnosis of adult forms of LGMD [10].

Cardiac involvement was rarely reported in MDC1A patients [31, 32]. The first comprehensive cardiac assessment in a series of MDC1A patients was reported by Spyrou et al. [33]. The authors noticed that the left ventricular ejection fraction in laminin $\alpha 2$ -negative children was significantly lower ($43\% \pm 11\%$) compared with laminin $\alpha 2$ -positive children ($53\% \pm 5\%$). In a subsequent review, Jones et al. [24] identified a range of electrocardiogram and echocardiogram abnormalities in 7 out of 20 patients (35%) with more than half being asymptomatic. In 2010, Germanyeh et al. [11] reported 5 patients with absent laminin $\alpha 2$ staining and cardiac abnormalities including mitral regurgitation, pulmonary hypertension, palpitations and wall motion hypokinesia on echocardiogram (Table 2). More recently, 3 patients (Table 2) have been described with cardiopathy: 2 with dilated cardiomyopathy, one of whom with ventricular arrhythmia [9] and one with impaired left ventricular contractility [34]. All of these reports combined with our patients (Patients #1 and #3) suggest that laminin $\alpha 2$ deficiency may indeed lead to an overt cardiomyopathy. To exclude any digenic phenomenon that would explain the presence of cardiac disease, we excluded

other cardiomyopathy causative genes including those associated with cardiac and skeletal muscle diseases.

From the genetic point of view, three of the mutations reported here were previously reported with a clear deleterious effect on laminin $\alpha 2$ chain when isolated or associated with other mutation in a compound heterozygous state (Table 2). The 8 pb insertion c.1854_1861dup identified in patients #3 and #4 was identified in 3 patients with 2 showing classical MDC1A presentation [19]. The homozygous c.1854_1861dup mutation (p.Leu621Hisfs*7) or the compound heterozygous c.1854_1861dup and c.7750-1713_7899-2153del (p.Ala2584Hisfs*8) induce a frameshift resulting in the total absence of laminin $\alpha 2$ in muscle. The c.2230C>T (p.Arg744*) homozygous mutation in patient #2 was previously reported in a brother and sister with similar presentation, although the sister was less severely affected [18]. The brother had a limb girdle muscular weakness starting at 15 years of age, severe contractures involving neck, elbows, ankles and knees; mild peripheral neuropathy, diffuse white matter abnormalities and normal cardiac evaluation at 39 years of age. Immunohistochemical and western blotting analyses disclosed partial laminin $\alpha 2$ chain deficiency. A smaller transcript arising from skipping of the exon containing the mutation and retaining the open reading frame was detected, thereby sustaining the presence of a partially functional laminin $\alpha 2$ chain. The other missense mutation identified in patients #3 and #4 (c.2461A>C, p.Thr821Pro) was already reported, associated with a missense variation (c.812C>T, p.Thr271Ile) at compound heterozygous state [34]. The patient had an exclusive central nervous system involvement showing among other things, refractory epilepsy associated with progressive cognitive regression since the age of 6, area of agyria in the occipital cortex on brain MRI, extensive white matter abnormalities with swelling and resulting widening of gyri. Surprisingly, there was normal muscular strength, tone and deep tendon reflexes, with the highest CK level being 1589 UI/L and no cardiac involvement. Laminin- $\alpha 2$ immunohistochemical studies revealed partial and irregular labelling [34]. The c.2461A>C, p.Thr821Pro was also reported in combination with an intronic mutation (c.5234 + 1G>A) in 2 patients: a Canadian one with classical MDC1A phenotype and cervical spine fusion, seizures and abnormal white matter (www.dmd.nl/) and in a Portuguese patient [34] (Table 2). In the latter, the phenotype was highly similar to the 2 brothers reported here with limb-girdle weakness, rigid spine syndrome, brain white matter abnormalities and impaired left ven-

tricular contractility, whereas the partial laminin $\alpha 2$ deficiency described in the third patient carrying the compound heterozygous c.1854_1861dup and missense mutation c.3832G>T, p.Gly1278Cys results from the production at the plasma membrane of a mutant form of laminin $\alpha 2$.

Beyond the classical MDC1A phenotype with absence of merosin expression [5], milder forms have been described in individuals with partial laminin $\alpha 2$ deficiency. In general, patients with absence of laminin $\alpha 2$ expression have an early presentation within the first months of life, typically do not acquire independent ambulation, usually require enteral feeding and ventilatory support [11]. By contrast, individuals with partial laminin $\alpha 2$ deficiency tend to have a milder form of CMD or may rarely have onset after infancy, delay in walking or proximal muscle weakness. They may show muscle pseudohypertrophy and/or rigid spine and are usually classified within the LGMD spectrum although they show characteristic white matter abnormalities [8, 10, 35–39]. Our 4 patients may be included in the milder group since they all had preserved ambulation at advanced ages and none of them required respiratory support. All these features are in agreement with the partial laminin $\alpha 2$ deficiency of variable severity uncovered by immunolabelling and western blot, patient #2 displaying the most pronounced deficiency. Theoretically, the 2 homozygous mutations observed in patients #1 and #2 should lead to absence of laminin $\alpha 2$ and thus to a classical MDC1A phenotype. Since laminin $\alpha 2$ is still present, even at low levels, one may expect that a posttranscriptional event such as an aberrant splicing which restores the *LAMA2* open reading frame as previously reported [19, 34]. For patients #3 and #4 the missense mutation in exon 18 may result in the translation of a protein carrying the mutated amino acid like previously described [34].

Taken together, these observations suggest that the diagnosis of laminin $\alpha 2$ deficient related diseases should be considered in adult patients presenting a myopathy with joint contractures and brain abnormalities (either white matter changes or structural abnormalities) or even cardiomyopathy. Since the cardiomyopathy may appear later in life, regular monitoring of cardiac function and electrocardiogram are required in these patients. Moreover, in atypical forms of LGMD without definite molecular diagnosis, brain MRI can offer a diagnostic clue either to suspect *LAMA2* as a candidate gene or to contribute in the validation of *LAMA2* mutations since NGS will be used more frequently for molecular genetic assessment of LGMDs.

Table 2
Previous reports of patients with LAMA2 mutations either identified in our patients or with overt cardiac disease

Ref.	Patient #	LAMA2 mutations / mRNA study	Overall phenotype		Cardiac involvement		Other features
			Laminin α2 staining	MDCIA	NI	NI	
[19]	5	Homozygous: c.1854_1861dup, p.Leu621His ^{s*} / mRNA: frameshift	Absent	MDCIA	NI	NI	No cognitive delay, no seizures. Brain MRI = white matter changes and no cortical abnormalities
[19]	11	c.1854_1861dup, p.Leu621His ^{s*} / c.3832G>T, p.Gly1278Cys / mRNA: frameshift	Partial deficiency	Slowly progressive Spastic paraparesis MDCIA	NI	NI	Slight cognitive delay, no seizures. Brain MRI = white matter changes and no cortical abnormalities
[19]	12	c.1854_1861dup, p.Leu621His ^{s*} / exon 56 deletion (c.7750-1713_7899-2153del) / mRNA: frameshift	Absent	MDCIA	NI	NI	No cognitive delay, no seizures. Brain white matter changes and no cortical abnormalities
[18]	Brother and sister	Homozygous: c.2230C>T, p.Arg744* / skipping of exon 15	Partial deficiency	LGMD with contractures	Absent at 39 years	Absent at 39 years	Mild peripheral neuropathy revealed by somatosensory brainstem evoked potentials testing. Brain MRI = diffuse white matter abnormalities in periventricular and subcortical areas
[34]	1	c.2461A>C, p.Thr821Pro / c.812C>T, p.Thr271Ile / No splicing defects in the vicinity of the mutations	Partial deficiency	Exclusive central nervous system involvement (cognitive impairment and refractory epilepsy) MDCIA	Absent	Absent	Macrocephaly, refractory epilepsy with progressive cognitive regression, bilateral divergent strabismus, normal muscular strength, tone and deep tendon reflexes, highest CK level 1589 U/L, area of agryria in the occipital cortex on brain MRI, extensive white matter abnormalities and swelling and widening of gyri. Normal motor function. Cervical spine fusion, seizures. Brain MRI = white matter abnormalities. Brain white matter abnormalities
LDB	2	c.2461A>C, p.Thr821Pro / c.5234+1G>A, p.Val1765Ser ^{s*} 21	NI	Rigid spine syndrome and limb-girdle weakness. MDCIA	NI	NI	Normal motor function. Cervical spine fusion, seizures. Brain MRI = white matter abnormalities. Brain white matter abnormalities
[34]	2	c.2461A>C, p.Thr821Pro / c.5234+1G>A, p.Val1765Ser ^{s*} 21	Partial deficiency	Rigid spine syndrome and limb-girdle weakness. MDCIA	Impaired left ventricular contractility	Impaired left ventricular contractility	Brain white matter abnormalities
[11]	38	c.4038T>G, p.Tyr1345*	Absent	Myopathy	Wall hypokinesia on echocardiogram	Wall hypokinesia on echocardiogram	Ventilatory support, scoliosis, mild contractures, ophthalmoplegia, enteral feeding at 10y, typical MDClA. White matter change on brain MRI.
[9, 18]	-	homozygous c.4405T>C, p.Cys1469Arg / c.4645C>T, p.Arg1549*	Partial deficiency	Myopathy resembling Inclusion Body Myositis MDClA	Dilated cardiomyopathy, ventricular arrhythmia (at 13 years)	Dilated cardiomyopathy, ventricular arrhythmia (at 20 years)	Mild wasting proximal leg muscles, calf hypertrophy, mild weakness all leg muscles; decreased knee reflexes, ankle reflexes brisk without other pyramidal signs; pes cavus, leukoencephalopathy
[11]	44	c.7881T>G, p.His2627Gln, homozygous	Absent	Myopathy	Wall hypokinesia on echocardiogram	Wall hypokinesia on echocardiogram	Decreased lung capacity (<70%FVC), scoliosis, mild contractures, feeding problems

LDB: Leiden muscular dystrophy pages database, NI: not investigated.

In addition, all dystrophic muscle biopsies, regardless of clinical phenotype, should be studied with multiple antibodies against different regions of laminin $\alpha 2$ using immunohistochemistry or western blot to detect *LAMA2* partial defects. This report also draws attention to the potential overlap between the 2 most common forms of CMD: MDC1A caused by the deficiency of laminin $\alpha 2$ chain and collagen VI-related muscular dystrophies.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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