Laminin α2 Deficiency-Related Muscular Dystrophy Mimicking Emery-Dreifuss and Collagen VI related Diseases

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Abstract

Background: Laminin α2 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 α2 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
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 α2 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 α2 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 α2 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, electromyography 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 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- and γ-sarcoglycans, dysferlin, α-dystroglycan, desmin, αB-crystallin, myotilin, myosin heavy chain, caveolin 3 and collagen VI. Laminin α2 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.
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 α2 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 α2 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 fibroblast 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 contractions including Achilles tendons (lengthened at 15 and 25 years old) and finger flexors requiring surgical repair at 25. At 25 years of age, a 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 MRE 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 semitendinosus, the semimembranosus 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 20's. 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 gracillis, 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 | Age at last walking | First symptoms | Maximal CK level | Muscle weakness | Joint involvement | Fatty involvements on muscle | Epilepsy (age at onset) | Brain imaging | Cardiac abnormalities | Other features |
|------|------------------------|---------------------|---------------|------------------|----------------|------------------|---------------------------|-----------------------|--------------|---------------------|---------------|
| 1    | 35                      | 6                   | Calves myalgia | WI at 1           | No             | No               | Prox (LL+UL), | No                     | Normal    | DCM, 80             | Calf hypertrophy |
| 2    | 32                      | 3                  | Severe hypotonia | WI at 1           | No             | No               | Prox (UL+LL), | No                     | Normal    | Polymicrogyria      | Carbamazepine |
| 3    | 49                      | 6                  | Spine and elbows stiffness | WI at 1       | No             | No               | Prox (LL+UL), | Normal    | DCM, 52             | Quadriplegia   |
| 4    | 47                      | 2.5                | Delayed walking | WI at 2.5        | No             | No               | Prox (LL+UL), | No                     | Normal    | PD     | -                  | -              |

A: ankles; AL: adductor longus muscle; BH: biceps brachii muscle; CK: creatine kinase; DCM: dilated cardiomyopathy; E: elbows; ENMG: electrophysiological findings; FF: finger flexors; FVC: forced vital capacity; GM: gastrocnemius medial head; 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; PRS: pseudo-rigidity; PS: psoas muscle; RF: rectus femoris muscle; SA: sartorius muscle; SH: shoulder; UL: upper limbs; V: 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, gastrocnemius 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 abnor-

mal brain MRI that showed leukencephalopathy. In addition, patient #4 developed several episodes of loss of consciousness with eye rolling and postictal amnesia, unresponsiveness preceded by blurred vision, parasthesia 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. Oph-
thalmologic examination, skin and rectal biopsies, atrylsulfatase, 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 elec-
troneuromyographic studies, performed in adulthood, showed normal nerve conduction studies and myo-
genic pattern at needle electromyography.

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 pat-
tern (data not shown). Routine immunohistocherical 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 nor-
mal 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 SGCC (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 cardiomy-
opathies 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 α2 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 car-
rried the c.2230C>T mutation in a heterozygous state. In light of these LAMA2 mutations and of the phe-
notypic 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 com-
 pound 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 α2 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 μm. (B) Immunostaining on muscle cryosections from a control individual (CT), patient #1 and patient #2. Three antibodies against different regions of the α2 chain of laminin were used: 4H8-2, NCL, and MAB1922. Bar: 50 μm. (C) Western blot analysis of muscle biopsies from patients #1–3, using MAB1922 antibody with densitometry values below the blot.
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 polymicrogyria. 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 α2 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 α2 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 α2-deficient CMD were already pointed out previously, indicating that partial laminin α2 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 α2-negative children was significantly lower (43% ± 11%) compared with laminin α2-positive children (53% ± 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, Germanych et al. [11] reported 5 patients with absent laminin α2 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 arrhythmias [9] and one with impaired left ventricular contractility [34]. All of these reports combined with our patients (Patients #1 and #3) suggest that laminin α2 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 causing 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 α2 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.Leu621Hesfs*7) or the compound heterozygous c.1854_1861dup and c.7750-1713_7899-2153del (p.Alu2584Hesfs*8) induce a frameshift resulting in the total absence of laminin α2 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 α2 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 α2 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 result-

tricular contractility, whereas the partial laminin α2 deficiency described in the third patient carrying the compound heterozygous c.1854_1861dup and missense mutation c.5832G>T, p.Gly1278Cys results from the production at the plasma membrane of a mutant form of laminin α2.

Beyond the classical MDC1A phenotype with absence of merosin expression [5], milder forms have been described in individuals with partial laminin α2 deficiency. In general, patients with absence of laminin α2 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 α2 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 α2 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 α2 and thus to a classical MDC1A phenotype. Since laminin α2 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 α2 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.
| Ref | Patient # | LAMA2 mutations (mRNA study) | Laminin α2 Overall | Cardiac involvement | Other features |
|-----|------------|-------------------------------|--------------------|--------------------|---------------|
| [19] 5 | Homozygous: c.1854_1861dup, Absent MDC1A | NI | No cognitive delay, no seizures. Brain MRI = p.Leu621Hisfs*7 | mRNA: white matter changes and no cortical frameshift abnormalities |
| [19] 11 | c.1854_1861dup, p.Leu621Hisfs*7 / Partial deficiency | Slowly | NI | Slight cognitive delay, no seizures. Brain MRI = c.3832G>T, p.Gly1278Cys / mRNA: progressive white matter changes and no cortical spastic abnormalities.paraparesis |
| [19] 12 | c.1854_1861dup, p.Leu621Hisfs*7 / Absent MDC1A | NI | No cognitive delay, no seizures. Brain white exon 56 deletion (c.7750-1713_7899-2153del) / mRNA: white matter changes and no cortical abnormalities |
| [18] Brother and sister | Homozygous: c.2230C>T, Partial deficiency | LGMD with Absent at 39 years | Mild peripheral neuropathy revealed by somatosensory brainstem evoked potentials, Brain MRI = diffuse white matter abnormalities in periventricular and subcortical areas |
| [34] 1 | c.2461A>C, p.Thr821Pro / Partial deficiency | Exclusive | Absent Macrocephaly, refractory epilepsy with c.812C>T, p.Thr271Ile / No splicing mutations involvement tone and deep tendon reflexes, highest CK level (cognitive 1589 UI/L, area of agyria in the occipital cortex impairment and on brain MRI, extensive white matter swelling and widening of gyri. Normal motor function. |
| [11] 2 | c.2461A>C, Partial deficiency | Rigid spine | Impaired left ventricular contractility Brain white matter abnormalities, c.5234 + 1 G>A, syndrome and p.Val1765Serfs*21 limb-girdle weakness. |
| [11] 38 | c.4035T>G, p.Tyr1345*, Absent MDC1A | Wall hypokinesia on echocardiogram | Ventilatory support, scoliosis, mild contractures, feeding problems homozygous (at 20 years) |
| [9, 18] – c.4405T>C, p.Cys1469Arg / Partial deficiency | Myopathy Dilated cardiomyopathy, ventricular arrhythmia 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 resembling Inclusion Body Myositis |
| [11] 44 | c.7881T>G, p.His2627Gln, Absent MDC1A | Wall hypokinesia on echocardiogram | Decreased lung capacity (<70%FVC), scoliosis, mild contractures, feeding problems homozygous (at 20 years) |

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

[1] Sparks S, Ojajärvi-Roy S, Harper A, Rutkowski A, Gordon E, Hoffman EP, et al. Congenital muscular dystrophy: overview. 2011.
[2] Banker BO. The congenital muscular dystrophies. In: Engel AG, Franzini-Amstrong C, editors. Myology. 2. 2nd ed. New York: McGraw-Hill; 1994. p. 1275-89.
[3] Tome FM. The Peter Emil Becker Award lecture 1998. The saga of congenital muscular dystrophy. Neuropediatrics. 1999;30(2):65-8.
[4] Bonnemann CG, Wang CH, Ojajärvi-Roy S, Deconinck N, Bertiini E, Ferreiro A, et al. Diagnostic approach to the congenital muscular dystrophies. Neuromuscular Disord. 2014;24(4):289-311.
[5] Tome FM, Evangelista T, Leclerc A, Sunada Y, Manole E, Estrament B, et al. Congenital muscular dystrophy with merosin deficiency. Comptes Rendus de l’Académie Des Sciences. 1994;317(4):351-7.
[6] Armourley M. The laminin family. Cell Adhesion & Migration. 2011;5(1):48-55.
[7] Norwood FL, Frieling C, Chinony PF, Eagle M, Dudley K, Straub V. Prevalence of genetic muscular disease in Northern England: In-depth analysis of a muscle clinic population. Brain. 2009;132(Pt 11):3175-86.
[8] Hermann R, Straub V, Meyer K, Kahl T, Wagner M, Voit T. Congenital muscular dystrophy with laminin alpha2 chain deficiency: Identification of a new intermediate phenotype and correlation of clinical findings to muscle immunohistochemistry. European Journal of Pediatrics. 1996;155(11):968-76.
[9] Carboni N, Mannina G, Ponzio M, Manfreda A, Solla E, Cocco E, et al. Dilated cardiomyopathy with conduction defects in a patient with partial merosin deficiency due to mutations in the laminin-alpha2-chain gene: A chance association or a novel phenotype? Muscle & Nerve. 2011;44(5):628-8.
[10] Guassonne BF, Carboni N, Nielsen JF, Danansee ER, Thomson C, Srenstorp K, et al. Clinical and molecular characterization of limb-girdle muscular dystrophy due to LAMA2 mutations. Muscle & Nerve. 2011;44(5):703-9.
[11] Goramayuth F, Clement E, Peng LH, Sorey J, Pagan J, Mein R, et al. Genotype-phenotype correlation in a large population of muscular dystrophy patients with LAMA2 mutations. Neuromuscul Disord. 2011;21(6):341-50.
[12] Dubowitz V. Muscle Biopsy: A practical approach. Second edition. Eastbourne: I, Tindall B, editors. 1985.
[13] Manial S, Ricas D, Sorey CA, Hoeldtzein M, Lleve S, Letourcq F, et al. Mutations in Emery-Dreifuss muscular dystrophy and their effects on emerin protein expression. Hum Mol Genet. 1998;7(5):855-64.
[14] Deconinck N, Doulal F, Lleve S, Barbos IC, Ricas D, Peccate C, et al. Protein- and mRNA-based phenotyp-genotype correlations in DMD/BMD with point mutations and molecular basis for BMD with nonsense and frame-shift mutations in the DMD gene. Human Mutation. 2007; 28(2):19-95.
[15] Deconinck N, Richard P, Alamban V, Belin A, Laforet P, Ferreiro A, et al. Bethlem myopathy: Long-term follow-up identifies COL6 mutations predicting severe clinical evolution. J Neurol Neurosurg Psychiatry. 2014.
[16] Abecasis GR, Cherny SS, Cookson WO, Cardon LR. Merlin-rapid analysis of dense genetic maps using sparse gene flow trees. Nat Genet. 2002;30(1):97-101.
[17] Nectoux J, de C, Boulain S, Letourcq F, Uriuibeera J, Penisson-Beumer I, et al. Detection of TRIM32 deletions in LGMD patients analyzed by a combined strategy of CGH array and massively parallel sequencing. Eur J Hum Genet. 2014.
[18] Di Blasi C, Frey E, Morandi L, Corbelio F, Guicheney P, Moru M. Mild muscular dystrophy due to a nonsense mutation in the LAMA2 gene resulting in exon skipping. Brain. 2001;124(Pt 4):689-704.
[19] Oliveira J, Santos R, Soares-Silva I, Jorge P, Vieira E, Oliveira M, et al. LAMA2 gene analysis in a cohort of 26 congenital muscular dystrophy patients. Clin Genet. 2008;74(5):502-12.
[20] Frederic MV, LaLande M, Boileau C, Hamroun D, Claustres M, Benoist C, et al. UMD-predictor, a new prediction tool for nucleotide substitution pathogenicity – application to four genes FBXN, FGFBP2, TGFBR1, and TGFBR2. Human Mutation. 2009;30(6):952-9.
[21] Fandras M, Tomé FMS, Heilbronn-Leclerc A, Evangelista T, Onulam A, Chevallay M, et al. Dystrophic muscular congenital confection with splenomegaly: Analyse clinicale,
histopathologique, immunocytochimique et génétique. Rev Neurol Paris. 1996;152:11-9.

[22] Quijano-Roy S, Sparks S, Rutkowski A. LAMA2-related muscular Dystrophy. 2012.

[23] Lokken N, Born AP, Dano M, Vissing J. LAMA2-related myopathy; frequency among congenital and limb-girdle muscular dystrophies. Muscle & Nerve. 2015.

[24] Jones K, Morgan G, Johnston H, Tribis V, Ooster RA, Wilkinson L, et al. The expanding phenotype of laminin alpha2 chain (merosin) abnormalities: Case series and review. Journal of Medical Genetics. 2001;38(10):649-57.

[25] Pan A, Merlini L, Tome FM, Chevallay M, Gobbi G. Merosin-negative congenital muscular dystrophy: occipital epilepsy with periodic spasms and focal cortical dysplasia. Report of three Italian cases in two families. Brain and Development. 1996;18:316-22.

[26] Martiniello F, Angelini C, Tervisian CP. Congenital muscular dystrophy with partial merosin deficiency and late onset epilepsy. European Neurology. 1998;40(1):37-45.

[27] Eymard B, Ferrerio A, Ben Youssef, M. Muscle diseases with prominent joint contractures: Main entities and diagnostic strategy. Rev Neurol (Paris). 2013;169(8-9):546-63.

[28] Tervisian CP, Martiniello F, Ferrazzini E, Angelini C. Divergence of central nervous system involvement in 2 Italian sisters with congenital muscular dystrophy, a clinical and neuroradiological follow-up. Eur Neurol. 1995;35:20-5.

[29] Philpot J, Cozan F, Pramock J, Sever C, Dubowitsir V, Bylander G, et al. Merosin-deficient congenital muscular dystrophy: The spectrum of brain involvement on magnetic resonance imaging. Neuromuscul Disord. 1999;9:81-5.

[30] Vigliano P, Dassi P, Di Biasi C, Mora M, Join L, LAMA2 stop-codon mutation: Merosin-deficient congenital muscular dystrophy with occipital polymicrogyria, epilepsy and psychomotor regression. Eur J Paediatr Neurol. 2009;13(1):72-6.

[31] Philpot J, Comi GP, Rigolotto C, Turconi C, Felisari G, Ciceri P, et al. An atypical case of partial merosin deficiency congenital muscular dystrophy. J Neurol. 1997;244(6):391-5.

[32] Sewry CA, Philpot J, Sorokin IM, Wilson LA, Naom I, Goodwin F, et al. Diagnosis of merosin-laminin 2 deficiency congenital muscular dystrophy by skin biopsy. The Lancet. 1996;347:582-4.

[33] Spyrou N, Philpot J, Foulie R, Camici PG, Muntoni F. Evidence of left ventricular dysfunction in children with merosin-deficient congenital muscular dystrophy. American Heart Journal. 1998;136(4):474-6.

[34] Marques J, Duarte ST, Costa S, Jacinto S, Oliveira J, Oliveira ME, et al. Atypical phenotype in two patients with LAMA2 mutations. Neuromuscul Disord. 2014;24(5):419-24.

[35] Hayashi YK, Ishihara T, Domen K, Hori H, Azuma K. A benign allelic form of laminin α2 chain deficient muscular dystrophy. Lancet. 1997;349:1147.

[36] Cohn RD, Herrmann R, Sorokin L, Wierer UM, Voci T. Laminin alpha2 chain-deficient congenital muscular dystrophy: Variable expression in severe and mild cases. Neurology. 1998;51:84-101.

[37] Naom I, D’Alessandro M, Sewry C, Philpot J, Munzon AV, Dubowitsir V, et al. Laminin α2 chain gene mutations in two siblings presenting with limb-girdle muscular dystrophy. Neuromuscul Disord. 1998;8:495-501.

[38] He Y, Jones KJ, Vignier, N., Morgan, G., Chevallay, M., Bansis, A., Estournet-Mathiaud, B., Heiz, H., Minus, T., Toml, F.M.S., North, K.N. and Quigley, P. Mild Congenital muscular dystrophy with primary partial laminin α2 chain deficiency: Molecular study. Neurology. 2001.

[39] Rajakulendran S, Parson M, Holton JL, Hannah MG. Clinical and pathological heterogeneity in late-onset partial merosin deficiency. Muscle & Nerve. 2011;44(4):590-3.