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Case report

Exome sequencing detects compound heterozygous nonsense *LAMA2* mutations in two siblings with atypical phenotype and nearly normal brain MRI

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Abstract

LAMA2 mutations cause the most frequent congenital muscular dystrophy subtype MDC1A and a variety of milder phenotypes, characterized by total or partial laminin-α2 deficiency. In both severe and milder cases brain MRI invariably shows abnormal white matter signal intensity. We report clinical, histopathological, imaging and genetic data on two siblings with very subtle, and at first undetected, reduction in laminin-α2 expression, and brain MRI showing minor non-specific abnormalities. Clinical features in the female proband were characterized by muscle weakness involving neck and axial muscles, and pelvic girdle and distal lower limb muscles, reduced tendon reflexes and pes cavus. Clinical features in a younger brother were similar, and remained stable in both siblings during the follow up. Whole exome sequencing (WES) detected two heterozygous truncating *LAMA2* mutations. Brain MRI in combination with laminin-α2 immunohistochemistry might not be sufficient and WES might be the only means to reach a diagnosis.

Keywords: Laminin-α2; *LAMA2*; Congenital muscular dystrophy type 1A; WES.

1. Introduction

The autosomal recessive congenital muscular dystrophy type 1A (MDC1A) results from a variety of mutations, either missense, nonsense, deletions or splice site variants, in the *LAMA2* gene [1]. The *LAMA2* gene, comprising 65 exons, encodes the α2 chain subunit of laminin-2 (merosin). Generally, complete absence of the laminin α2 chain leads to a very severe disease course, while partial deficiency results in a variety of milder phenotypes [2,3]. Accordingly, skeletal muscles of MDC1A patients show, depending on complete or partial absence of the laminin-α2 chain, either severe dystrophic features such as muscle degeneration and regeneration, inflammation, atrophy and fibrosis, or milder myopathic features [4]. In MDC1A, in addition to skeletal muscle, other tissues are affected including brain, Schwann cells, heart and lung [5].

MDC1A is the most frequent subtype among congenital muscular dystrophies (CMD) [6]. Most common presenting symptoms are hypotonia at birth or in the first weeks of life or delayed motor milestones during the first year of age. Rare patients, among those with complete absence of laminin α2 protein on muscle biopsy, achieve independent ambulation; conversely, most patients, among those with partial laminin-α2 deficiency, achieve ambulation [3,6–10].

We report clinical, histopathological, imaging and genetic data on two siblings with very subtle reduction in laminin-α2

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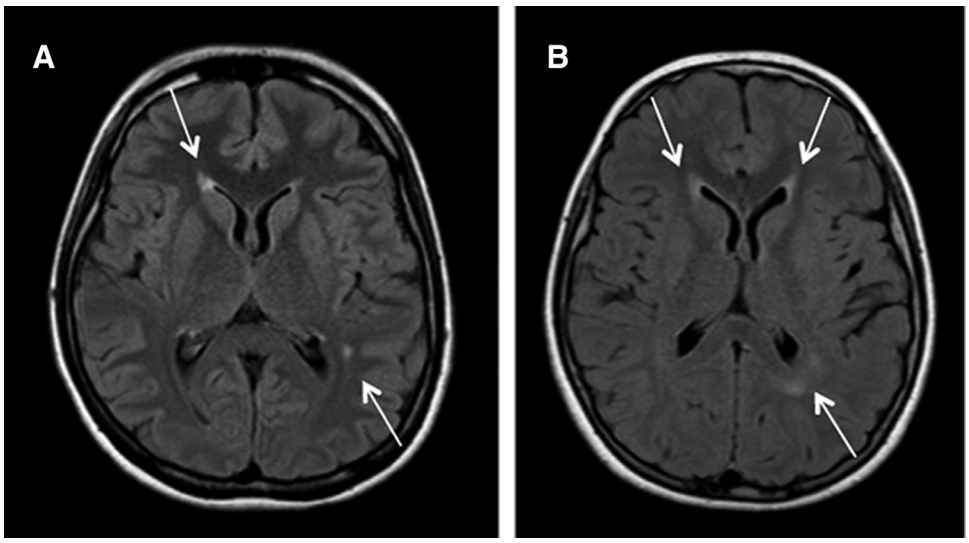

Fig. 1. Brain MRI. Axial FLAIR image of the proband (A) showing two small non-specific hyperintensities in the right frontal periventricular and in the left peritrigonal white matter (arrows); and of the brother (B) showing non-specific abnormalities in the frontal periventricular white matter bilaterally and in the left paratrigonal white matter (arrows).

expression in the proband muscle biopsy, unusual clinical features and normal brain MRI.

2. Case report

This study was performed according to institutional review board-approved clinical protocols. Parental informed consent was obtained for muscle biopsy and exome analysis.

The proband is a female, now 16 years old, born from non consanguineous Italian parents, presenting with congenital onset of bilateral clubfoot, hypotonia, hyperlaxity and mild motor development delay; autonomous gait was achieved at 18 months, and difficulties in climbing stairs and running were reported since her first years of age. At 10 years she underwent foot surgery for bilateral Achilles tendon lengthening and preoperative CK levels resulted markedly elevated (4000 U/l). At the first neurological examination, at age 13, she presented with overt muscle weakness involving neck and axial muscles, pelvic girdle (with positive Gowers sign) and distal lower limb muscles, reduced tendon reflexes and pes cavus; sensory examination was normal. No ocular or bulbar signs, and no cognitive impairment as well as cardiac or respiratory dysfunction were detected. Electroneuromyographic study showed normal nerve conduction values and a myopathic pattern in proximal muscle of upper and lower limbs.

A muscle biopsy of the quadriceps, taken at age 13, showed variable muscle fibre size with mostly hypertrophic and atrophic fibres, fibre splitting, central nuclei, but no necrotic or regenerating fibres and no fibrosis. Immunohistochemistry of dystrophin, dystrophin-associated proteins, α-dystroglycan, laminin $\alpha 2$, and Western blot of calpain 3 and dysferlin were all normal.

A younger brother, now 10 years old, showed the same phenotypical features, with axial, proximal and distal muscle weakness, calf hypertrophy and high CK levels (1000 U/l); another sister was healthy.

Brain MRI of the female at age 16 years was normal except for two minor signal abnormalities in the periventricular white matter, interpreted as non-specific changes by a neuroradiologist with extensive expertise in leukodystrophies. Subsequently, a brain MRI performed in the brother at age 10 years, showed similar subtle abnormalities in the white matter (Fig. 1). Myopathic features were unchanged in both siblings at last observation, at age 16 years and 10 years respectively, except for progressive Achilles tendon contractures in the boy, that required surgical lengthening. Cardiac evaluation continued to be normal in both patients at age 16 and 10 years, respectively.

Whole exome sequencing revealed in both siblings two compound heterozygous *LAMA2* mutations: c.4936G>T causing substitution of a glutamic acid with a stop codon (p.Glu1646*), previously reported [Nelson et al. 11], and c.8625delA, causing frameshift and insertion of an abnormal stop codon 27 amino acids downstream (p.Ala2876fs*27), never reported before. Sanger sequencing confirmed the mutations in both patients and showed that the former was inherited from the father and the latter from the mother. The c.8625delA mutation was not present in gnomAD (http://gnomad.broadinstitute.org/) and Exome Variant Server (http://genome.ucsc.edu/) data banks, and was predicted as disease causing, using the mutation taster software package.

Reassessment of laminin-$\alpha 2$ expression on the proband muscle biopsy after exome results, showed slightly reduced laminin-$\alpha 2$ expression, by immunohistochemistry using the commercial monoclonal antibody directed against the 80 kDa C-terminus of laminin-$\alpha 2$ (MAb1922; Chemicon, Temecula, California), and normal expression using the 300 kDa N-terminus (4H8-2; Alexis Biochemicals, San Diego, California) or the NCL-Merosin Clone Mer3/22B2 (Leica

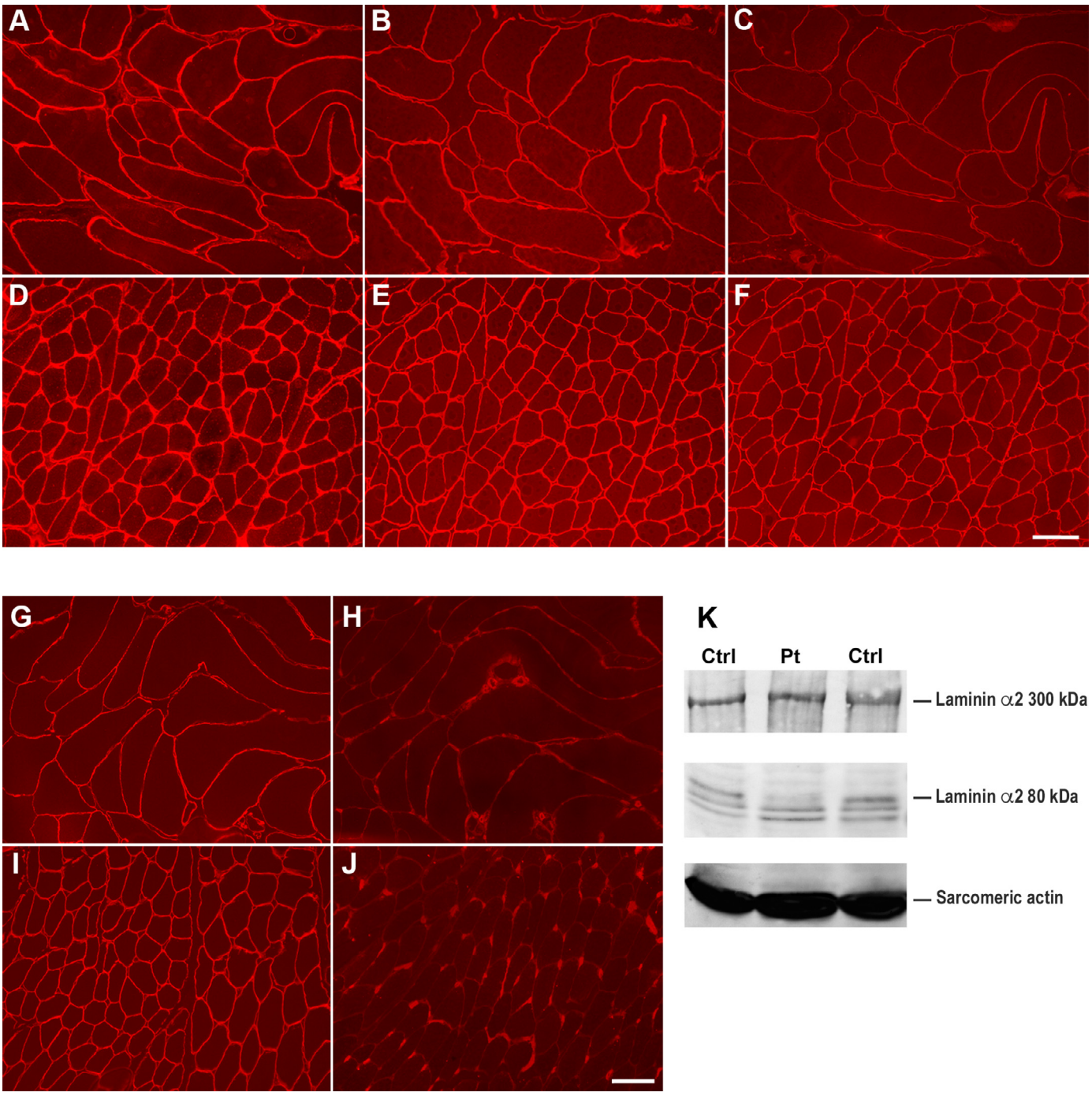

Fig. 2. (A-F) Immunohistochemistry on consecutive sections of patient 1 (A-C) and control (D-F) muscle with anti-dystrophin (A,D), anti-laminin $\alpha 2$ 300 kDa N-terminus (B,E) and anti-laminin $\alpha 2$ 80 kDa C-terminus (C,F) antibodies, showing almost normal laminin $\alpha 2$ expression in the patient. Bar = 100 μm. (G,I) Immunostaining of laminin-$\alpha 2$ with NCL-Merosin antibody Clone Mer3/22B2, and of laminin-$\alpha 5$ (H,J) showing similar expression of both proteins in patient 1 (G,H) and control (I,J) muscle. Bars = 100 μm. (K) Laminin $\alpha 2$ immunoblot from muscle of patient 1 and controls showing reduced intensity of the 80 kDa band in the patient. The two adjacent bands are unspecific bands always observed with this antibody and with the immunoblotting conditions used.

Microsystems, Wetzlar, Germany) antibodies (Fig. 2). By immunoblot the laminin-$\alpha 2$ 300 kDa band appeared normal, while the 80 kDa band was of greatly reduced intensity (Fig. 2). Laminin-$\alpha 5$ was faintly expressed on the fibre surfaces, and difficult to perceive as increased compared to a normal age-matched control (Fig. 2).

Amplification of skeletal muscle cDNA fragments across the two mutations showed two bands of the expected size. Sequencing revealed that both alleles carrying the two mutations were transcribed. Thus the mutations do not affect pre-mRNA splicing.

3. Discussion

Cases that show residual laminin-α2 expression are characterized by a more heterogeneous clinical phenotype than cases with total lack of laminin-α2 [9,12]. Clinical features range from ambulant patients with limb girdle muscular dystrophy and various degrees of central nervous system (CNS) involvement, to atypical phenotypes, showing almost exclusive CNS involvement or marked cardiac dysfunction, rigid spine syndrome and limb-girdle weakness [13] or myopathy resembling inclusion body myositis [14]. Furthermore, variable peripheral nerve involvement has been observed in *LAMA*2-mutated patients [15–17]. Most importantly, a range of brain abnormalities have been reported in all severe and milder forms of *LAMA*2 mutated patients [18,19]. Central nervous system involvement is invariably characterized by abnormal signal intensity in the supratentorial white matter and/or polymicrogyria [18–21]. The brain changes consist of white matter hypointensity on T1-weighted images and hyperintensity on T2-weighted images within the cerebral hemispheres.

In our proband brain MRI was normal, except for two minor non-specific abnormalities in the white matter. In the younger brother brain MRI showed similar white matter non-specific changes reminiscent also of the subtle abnormalities previously described in a girl with LGMD [22]. In *LAMA*2-mutated patients, normal brain MRI has been reported only in the neonatal period [3,8], but never, to the best of our knowledge, in later periods of life, not even in mildly affected adult patients except for a previous case reported by Nelson et al. [11], carrying the c.4936G>T mutation found in our two siblings. Nelson's case presented with infantile onset limb girdle myopathy and developed dilated cardiomyopathy in adult age, suggesting a diagnosis of EDMD. Our two siblings share with him some similar clinical features: presence of early joint contractures in Achilles tendon requiring lengthening and calf hypertrophy, but no cardiac involvement.

Other unusual features in our patients were the clinical presentation, more resembling congenital myopathy than muscular dystrophy, and the stable progression also typical of congenital myopathies, except for high CK levels.

Indeed the diagnosis in our patients was challenging as the partial deficiency of laminin-α2 was difficult to detect even when comparing the staining with a control cryosection on the same slide. Therefore, our patients have remained without genetic characterization for several years.

The partial expression of laminin-α2 is in line with the type of mutations, both allowing the production of a partially functional truncated protein. The p.Glu1646*, variant is likely to produce a protein lacking 1477 aminoacids, similarly to the patient with the same mutation, but in homozygosis, described by Nelson et al. [11] (patient 1), showing "slight irregularities and weakness of laminin-α2 staining". The explanation of the authors for such partial expression, unexpected from the type of mutation, was that "a posttranscriptional event such as an aberrant splicing which restores the *LAMA*2 open reading frame" could be expected, although no proof of that was provided. Unfortunately, by analysing the mRNA in our patient, we have not been able to demonstrate alternative splicing products, but found that both alleles carrying the two mutations were transcribed. In our patient at least one allele (the one carrying the c.8625delA variant) should allow the production of a truncated protein in which the epitope recognized by the 300 kDa antibodies is still present, in line, therefore, with the normal expression of laminin-α2 by immunohistochemistry and immunoblot using these antibodies. Although we could not find the precise epitope sequence of the 80 kDa antibody, it is known that it recognizes an epitope towards the C-terminus. The changes in the portion of the protein encoded by the allele carrying the c.8625delA which causes a frameshift and insertion of 27 new residues, could perhaps disturb the conformation of the epitope recognized by this antibody and therefore account for the reduced laminin-α2 intensity on fibre surface by immunohistochemistry and the residual 80 kDa band on immunoblot.

Although *LAMA*2 disease-associated variants are increasingly recognized as cause of muscular dystrophy, they are probably still underestimated. Furthermore, it has become clear that the clinical and histological presentations, as well as outcomes, may vary widely between subtypes and among different affected individuals. Brain MRI in combination with laminin-α2 immunohistochemistry, recently proposed [1] in the diagnostic work up, might not be sufficient to address molecular analysis, and WES might be the only means to reach a diagnosis.

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References

[1] Oliveira J, Gruber A, Cardoso M, Taipa R, Fineza I, Gonçalves A, et al. LAMA2 gene mutation update: Towards a more comprehensive picture of the laminin-α2 variome and its related phenotypes. Hum Mutat 2018;39(10):1314–37. doi:10.1002/humu.23599.

[2] Helbling-Leclerc A, Zhang X, Topaloglu H, Cruaud C, Tesson F, Weissenbach J, et al. Mutations in the laminin alpha 2-chain gene (LAMA2) cause merosin-deficient congenital muscular dystrophy. Nat Genet 1995;11(2):216–18.

[3] Geranmayeh F, Clement E, Feng LH, Sewry C, Pagan J, Mein R, et al. Genotype-phenotype correlation in a large population of muscular dystrophy patients with LAMA2 mutations. Neuromuscul Disord 2010;20(4):241–50. doi:10.1016/j.nmd.2010.02.001.

[4] Philpot J, Sewry C, Pennock J, Dubowitz V. Clinical phenotype in congenital muscular dystrophy: correlation with expression of merosin in skeletal muscle. Neuromuscul Disord 1995;5(4):301–5.

[5] Leivo I, Merosin Engvall E. a protein specific for basement membranes of Schwann cells, striated muscle, and trophoblast, is expressed late in nerve and muscle development. Proc Natl Acad Sci USA 1988;85:1544–8.

[6] Sframeli M, Sarkozy A, Bertoli M, Astrea G, Hudson J, Scoto M, et al. Congenital muscular dystrophies in the UK population: clinical and molecular spectrum of a large cohort diagnosed over a 12-year period. Neuromuscul Disord 2017;27(9):793–803. doi:10.1016/j.nmd.2017.06.008.

[7] Gavassini BF, Carboni N, Nielsen JE, Danielsen ER, Thomsen C, Svenstrup BelloL, et al. Clinical and molecular characterization of limb-girdle muscular dystrophy due to LAMA2 mutations. Muscle Nerve 2011;44(5):703–9. doi:10.1002/mus.22132.

[8] Oliveira J, Santos R, Soares-Silva I, Jorge P, Vieira E, Oliveira ME, et al. LAMA2 gene analysis in a cohort of 26 congenital muscular dystrophy patients. Clin Genet 2008;74(6):502–12. doi:10.1111/j.1399-0004.2008.01068.x.

[9] Tezak Z, Prandini P, Boscaro MM, Marin A, Devaney J, Marino M, et al. Clinical and molecular study in congenital muscular dystrophy with partial laminin alpha 2 (LAMA2) deficiency. Hum Mutat 2003;21(2):103–11. doi:10.1002/humu.10157.

[10] Løkken N, Born AP, Duno M, Vissing J. LAMA2-related myopathy: frequency among congenital and limb-girdle muscular dystrophies. Muscle Nerve 2015;52(4):547–53. doi:10.1002/mus.24588.

[11] Nelson I, Stojkovic T, Allamand V, Leturcq F, Bécane HM, Babuty D, et al. Laminin $\alpha 2$ deficiency-related muscular dystrophy mimicking emery-dreifuss and collagen VI related diseases. J Neuromuscul Dis 2015;2(3):229–40. doi:10.3233/JND-150093.

[12] Pegoraro E, Marks H, Garcia CA, Crawford T, Mancias P, Connolly AM, et al. Laminin alpha2 muscular dystrophy: Genotype/phenotype studies of 22 patients. Neurology 1998;51(1):101–10.

[13] 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. doi:10.1016/j.nmd.2014.01.004.

[14] Di Blasi C, Mora M, Pareyson D, Farina L, Sghirlanzoni A, Vignier N, et al. Partial laminin alpha2 chain deficiency in a patient with myopathy resembling inclusion body myositis. Ann Neurol 2000;47(6):811–16.

[15] Shorer Z, Philpot J, Muntoni F, Sewry C, Dubowitz V. Demyelinating peripheral neuropathy in merosin-deficient congenital muscular dystrophy. J Child Neurol 1995;10(6):472–5. doi:10.1177/088307389501000610.

[16] Quijano-Roy S, Renault F, Romero N, Guicheney P, Fardeau M, Estournet B. EMG and nerve conduction studies in children with congenital muscular dystrophy. Muscle Nerve 2004;29(2):292–9. doi:10.1002/mus.10544.

[17] Di Muzio A, De Angelis MV, Di Fulvio P, Ratti A, Pizzuti A, Stuppia L, et al. Dysmyelinating sensory-motor neuropathy in merosin-deficient congenital muscular dystrophy. Muscle Nerve 2003;27(4):500–6. doi:10.1002/mus.10326.

[18] Lamer S, Carlier RY, Pinard JM, Mompoint D, Bagard C, Burdairon E, et al. Congenital muscular dystrophy: Use of brain MR imaging findings to predict merosin deficiency. Radiology 1998;206(3):811–16. doi:10.1148/radiology.206.3.9494506.

[19] Philpot J, Cowan F, Pennock J, Sewry C, Dubowitz V, Bydder G, Muntoni F. Merosin-deficient congenital muscular dystrophy: the spectrum of brain involvement on magnetic resonance imaging. Neuromuscul Disord 1999;9(2):81–5.

[20] Vigliano P, Dassi P, Di Blasi C, Mora M, Jarre 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. doi:10.1016/j.ejpn.2008.01.010.

[21] Sunada Y, Edgar TS, Lotz BP, Rust RS, Campbell KP. Merosin-negative congenital muscular dystrophy associated with extensive brain abnormalities. Neurology 1995;45(11):2084–9.

[22] Chan SH, Foley AR, Phadke R, Mathew AA, Pitt M, Sewry C, Muntoni F. Limb girdle muscular dystrophy due to LAMA2 mutations: diagnostic difficulties due to associated peripheral neuropathy. Neuromuscul Disord 2014;24(8):677–83. doi:10.1016/j.nmd.2014.05.008.