

Short Report

LAMA2 gene analysis in a cohort of 26 congenital muscular dystrophy patients

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Congenital muscular dystrophy type 1A (MDC1A) is caused by mutations in the *LAMA2* gene encoding laminin- $\alpha 2$. We describe the molecular study of 26 patients with clinical presentation, magnetic resonance imaging and/or laminin- $\alpha 2$ expression in muscle, compatible with MDC1A. The combination of full genomic sequencing and complementary DNA analysis led to the particularly high mutation detection rate of 96% (50/52 disease alleles). Besides 22 undocumented polymorphisms, 18 different mutations were identified in the course of this work, 14 of which were novel. In particular, we describe the first fully characterized gross deletion in the *LAMA2* gene, encompassing exon 56 (c.7750-1713_7899-2153del), detected in 31% of the patients. The only two missense mutations detected were found in heterozygosity with nonsense or truncating mutations in the two patients with the milder clinical presentation and a partial reduction in muscle laminin- $\alpha 2$. Our results corroborate the previous few genotype/phenotype correlations in MDC1A and illustrate the importance of screening for gross rearrangements in the *LAMA2* gene, which may be underestimated in the literature.

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Congenital muscular dystrophy type 1A (MDC1A) is the most frequent form of congenital muscular dystrophy (CMD) in the European population, accounting for 30–50% of the cases (1–3). Major clinical symptoms include muscle weakness, raised serum creatine phosphokinase, no independent ambulation, cerebral white matter abnormalities and, in the most severe cases, respiratory insufficiency, which often leads to death in early childhood (4).

The *LAMA2* gene (MIM#156225), consisting of 65 exons and a 9.5-kb open reading frame, is implicated in MDC1A (5, 6). Two alternative transcripts have been described (accession numbers NM_000426 and NM_001079823); both isoforms are functional and encode the $\alpha 2$ chain of laminin-211 ($\alpha 2$ - $\beta 1$ - $\gamma 1$), laminin-221 ($\alpha 2$ - $\beta 2$ - $\gamma 1$) and laminin-213 ($\alpha 2$ - $\beta 1$ - $\gamma 3$) [nomenclature reviewed in (7)]. Laminin-211 trimer is predominantly expressed in skeletal muscle, cerebral white matter and Schwann cells and is a major component of the basal membrane (8). It binds to α -dystroglycan and the integrin- $\alpha 7$ - $\beta 1$ complex, establishing a connection between the cytoskeleton and the extracellular matrix [reviewed in (9)].

MDC1A patients usually have complete absence of laminin- $\alpha 2$ staining in muscle biopsies associated with the presence of truncating mutations. Patients presenting partial deficiency of laminin- $\alpha 2$ may also have mutations in *LAMA2*, or this deficit may be secondary to mutations in other genes acting in the α -dystroglycan glycosylation pathway [reviewed in (10)].

To date, 94 distinct mutations have been reported in the *LAMA2* locus-specific database (LSDB) (<http://www.lovd.nl/LAMA2>), the majority of which are small out-of-frame deletions (31.9%) and nonsense mutations (29.8%). Others include splice mutations (16.0%), missense substi-

tutions (14.9%) and small duplications (7.4%). There is no noticeable mutational hotspot.

Since the identification of the *LAMA2* gene, a few reports have described a significant number of patients with mutations (11–14). However, the mutation detection rates were lower than could perhaps be expected considering the clinical presentation and/or total absence of laminin- $\alpha 2$ in muscle, used as selection criteria. This has been attributed to factors such as technical difficulties inherent to mutation screening in large genes, the inclusion of patients with other clinically indistinguishable forms of CMD, or the existence of heterozygous deletions/duplications that are not detected by standard polymerase chain reaction (PCR)-based screening methods. This study describes the molecular characterization of a group of 26 MDC1A patients. A total of 18 distinct *LAMA2* mutations were detected corresponding to 96% of the disease alleles. In particular, a single new large exonic deletion, which accounted for 10 disease alleles, is described in detail.

Materials and methods

Patients

The study included a total of 26 CMD cases from Portugal (patients 1–11, 13–17, and 22–26), Spain (patients 12 and 18–20) and Switzerland (patient 21) who met defined clinical criteria for MDC1A, compatible magnetic resonance imaging (MRI) anomalies and/or revealed changes in muscle laminin- $\alpha 2$ immunostaining (Table 1). The parents were screened, whenever possible, to confirm mutation allelism or homozygosity. None of the families were consanguineous. Informed consent was obtained for the molecular studies.

Table 1. Clinical-pathological data of the congenital muscular dystrophy patients

Patient	Sex	Age	Age of onset	Clinical presentation	Highest CPK (U/l)	Pattern/ progression of weakness	Best motor achievement	Contractures	Central nervous system involvement/ seizures	Magnetic resonance imaging	Laminin- $\alpha 2$ in muscle ^a
1	M	19 years	At birth	Hypotonia and feeding problems	1410	Severe generalized weakness and scoliosis	No motor milestones achieved	All joints	No cognitive delay and no seizures	White matter changes and no gyral abnormalities	Total absence
2	M	7 years	At birth	Hypotonia and poor spontaneous movements	2697	Generalized weakness with proximal predominance	Assisted trunk control	Knees and hips and limited ankle movements	No cognitive delay and no seizures	White matter changes and no gyral abnormalities	Total absence
3	F	7 years ^b	4 months	Hypotonia and areflexia	3265	Proximal weakness	Sat unsupported	No	No cognitive delay and no seizures	White matter changes and abnormal bilateral frontal gyration	Total absence
4	M	7 years	At birth	Hypotonia and feeding problems	1650	Generalized weakness with axial and proximal predominance and improvement in motor function	Sat unsupported	No	No cognitive delay and no seizures	White matter changes and abnormal occipital gyration	Total absence
5	M	7 years	At birth	Hypotonia	1156	Muscular weakness with facial and bulbar paresis and slight improvement in motor function	Sat unsupported	Knees	No cognitive delay and no seizures	White matter changes and no gyral abnormalities	Total absence
6	F	17 years	At birth	Hypotonia and arthrogryposis	nd	Generalized weakness with axial predominance	Good cephalic control and sat unsupported	Knees	No cognitive delay and no seizures	White matter changes and no gyral abnormalities	Total absence
7	M	13 years	2 months	Generalized hypotonia	840	Proximal weakness with mild progression, facial and bulbar paresis, severe scoliosis (>9 years), and nocturnal ventilation (>3 years)	Cephalic control and sat unsupported	Generalized	No cognitive delay and no seizures	White matter changes and no gyral abnormalities	Total absence
8	M	4 years	At birth	Hypotonia and neonatal asphyxia	3782	Muscular weakness with axial and proximal predominance	Cephalic control and moves hands towards and manipulates objects (not against gravity)	Bilateral <i>talipes equinus</i>	No apparent cognitive delay and no seizures	nd	Total absence

Table 1. Continued

Patient	Sex	Age	Age of onset	Clinical presentation	Highest CPK (U/l)	Pattern/ progression of weakness	Best motor achievement	Contractures	Central nervous system involvement/ seizures	Magnetic resonance imaging	Laminin- α 2 in muscle ^a
9	M	11 years	At birth	Hypotonia and feeding problems	nd	Muscular weakness with axial and proximal predominance and scoliosis	Cephalic control, trunk control with brace, and handwriting	Elbows and knees	No cognitive delay and no seizures	nd ^c	Total absence
10	M	7 years	At birth	Hypotonia and poor spontaneous movements	2697	Muscular weakness with axial and proximal predominance	Sat unsupported	Elbows and knees	No cognitive delay and no seizures	nd	Total absence
11	M	24 years	3 years	Spastic paraparesis	838	Slowly progressive spastic paraparesis	Spastic gait	No	Slight cognitive delay and no seizures	White matter changes and no gyral abnormalities	Partial absence
12	M	3 years	At birth	Hypotonia and mild neonatal distress	1085	Generalized axial weakness and respiratory distress episodes	Sat unsupported	No	No cognitive delay and no seizures	White matter changes and no gyral abnormalities	Total absence
13	M	Died at 9 months	At birth	Hypotonia, hyporeflexia and bilateral talipes equinus	nd	Generalized axial hypotonia, pectus excavatum, and respiratory distress episodes	No motor milestones achieved	No	No apparent cognitive delay and no seizures	nd	Total absence
14	F	2 years	At birth	Hypotonia and feeding and respiratory problems	1999	Muscular weakness with axial and proximal predominance	Sat unsupported	No	No cognitive delay and no seizures	White matter changes and no gyral abnormalities	nd
15	F	27 years	At birth	Hypotonia and arthrogryposis	nd	Proximal weakness and scoliosis	Sat unsupported	Elbows and knees	No cognitive delay and no seizures	White matter changes and no gyral abnormalities	Total absence
16	F	4 years	In utero	Hypotonia	4460	Muscular weakness with facial diparesis, scoliosis, severe muscle atrophy, and under BIPAP	Plays with hands	Generalized	No cognitive delay and no seizures	White matter changes and no gyral abnormalities	Total absence
17	M	2 years	At birth	Hypotonia and bilateral talipes equinus	3866	Muscular weakness with facial diparesis	Sat unsupported	No	No cognitive delay and no seizures	White matter changes and no gyral abnormalities	Total absence
18	F	7 years	At birth	Generalized hypotonia and areflexia	5080	Severe and generalized weakness, scoliosis, equinus varus feet, and under BIPAP	No trunk or cephalic control	Generalized	No cognitive delay and no seizures	White matter changes and no gyral abnormalities	Total absence

Table 1. Continued

Patient	Sex	Age	Age of onset	Clinical presentation	Highest CPK (U/l)	Pattern/ progression of weakness	Best motor achievement	Contractures	Central nervous system involvement/ seizures	Magnetic resonance imaging	Laminin-α2 in muscle ^a
19	M	20 years	At birth	Generalized hypotonia and areflexia	3264	Muscular weakness with axial and proximal predominance and scoliosis	Independent gait	Elbows and ankles	No cognitive delay and no seizures	White matter changes and no gyral abnormalities	nd
20	F	18 years ^b	2 years	Generalized hypotonia and areflexia	593	Generalized weakness and no scoliosis	Independent gait	No	No cognitive delay and no seizures	White matter changes and no gyral abnormalities	Partial absence
21	F	4 years	At birth	Hypotonia and hydronephrosis (left kidney) grade III–IV	6987	Scoliosis and plagiocephalus	Assisted trunk control	No	No cognitive delay and no seizures	White matter changes and no gyral abnormalities	Total absence
22	M	9 months	At birth	Hypotonia, poor spontaneous movements and feeding problems	4706	Muscular weakness with proximal predominance and slight improvement in motor function	Incomplete cephalic control	Elbows, knees and ankles	No apparent cognitive delay and no seizures	White matter changes and no gyral abnormalities	Total absence
23	F	2 years	At birth	Hypotonia and feeding and respiratory problems	1700	Muscular weakness with axial and proximal predominance	Sat unsupported	Elbows and knees	No cognitive delay and no seizures	White matter changes and no gyral abnormalities	Total absence
24	M	3 years	At birth	Hypotonia and feeding problems	1770	Muscular weakness with proximal predominance and hip congenital luxation	Cephalic control and assisted trunk control	Knees	No cognitive delay and no seizures	White matter changes and no gyral abnormalities	Total absence
25	F	3 months	<i>In utero</i>	Generalized hypotonia with proximal predominance and feeding problems	5530	Muscular weakness with axial and proximal predominance	No cephalic control	Knees and ankles	No apparent cognitive delay and no seizures	White matter changes and no gyral abnormalities	Total absence
26	M	1 year	<i>In utero</i>	Hypotonia and arthrogryposis (hands and feet)	5615	Severe muscular weakness with proximal predominance and facial diparesis	Sat unsupported	Knees	No apparent cognitive delay and no seizures	White matter changes and no gyral abnormalities	Total absence

BIPAP, bi-level positive airway pressure; CPK, creatine phosphokinase; F, female; M, male; nd, not done.

^aImmunohistochemical staining with NCL-merosin monoclonal antibody (Novocastra), recognizing the 300-kDa amino-terminus fragment.^bLost to follow-up (patient 3 at age 4 years and patient 20 at age 16 years).^cWhite matter changes observed on computerized tomography scan.^dTested in the neonatal period.

Genomic DNA analysis

Genomic DNA (gDNA) was extracted from peripheral blood by the salting-out method (15). All 65 exons of *LAMA2* were amplified by PCR using intronic M13-tailed primers. Amplicons were purified using ExoSAP-IT® (USB Corporation, Cleveland, OH) and sequenced with M13 universal primers and BigDye™ Terminator Cycle Sequencing Kit V1.1 (Applied Biosystems, Foster City, CA). Products were resolved on an ABI 3130xl Genetic Analyzer (Applied Biosystems). Mutation analysis was aided by SEQSCAPE V2.5 software (Applied Biosystems) using the complementary DNA (cDNA) reference sequence NM_000426.3. Population screening was carried out in 150 anonymized control samples by single-stranded conformation analysis (SSCA), direct sequencing or fragment size analysis.

Bioinformatics

The effect of splicing mutations was predicted with the aid of the GENSCAN program (<http://genes.mit.edu/GENSCAN.html>) using segments of the *LAMA2* genomic reference sequence NC_000006:129246035–129879404. The scores of normal and mutated splice sites were calculated according to Shapiro and Senapathy (16). For mutations predictably inducing amino acid alterations, phylogenetic conservation analysis was performed with laminin- α 2 protein sequences from several organisms (Data S1, supporting information online), aligned using the software CLUSTALX version 1.83 (17).

cDNA analysis

Total RNA was extracted from muscle or skin biopsies using the Versagene RNA Purification kit (Gentra, Minneapolis, MN). Reverse transcription-PCR (RT-PCR) was performed using Superscript One-Step RT-PCR with Platinum Taq (Invitrogen, Carlsbad, CA) and previously described primer sets (5). For the gross deletion, the specific primers designed to amplify exons 54–58 were c.54F – 5'GGTGTTACCAAAGGATGTTCCC3' and c.58R – 5'CAGCATTTTTTGAA-GGACACAGG3'. Products were sequenced as described above.

Haplotyping

Typing was performed using short tandem repeats (STRs) flanking the *LAMA2* locus: D6S1715, D6S407, D6S1620, D6S1705 and D6S1572; and

intragenic single nucleotide polymorphisms (SNPs): c.3174+38A>G, c.5466G>A, c.5502A>G, c.5727-24_5727-21delinsACTG, c.6237G>A, c.6707+37T>C, c.7760T>C, c.7830C>G, c.7845G>A and c.*190_192dupATA.

Southern blot analysis

gDNA from patients 1 and 12 was digested with *AccI* (New England Biolabs, Beverly, MA), electrophoresed on a 0.8% agarose gel and transferred to a GeneScreen Plus® membrane (Perkin Elmer, Waltham, MA). This was hybridized with a cDNA probe recognizing exons 54–58 labelled with fluorescein (Gene Images Random Prime Labelling Module, GE Life Sciences, Piscataway, NJ). After antibody incubation, the membrane was washed at 60°C once in 1× SSC/0.1% SDS and twice in 0.5× SSC/0.1% SDS for 15 min each and developed using CDP-Star detection reagent (GE Life Sciences).

Long-range and deletion-specific PCR

Exon 56 deletion breakpoints were determined by amplification of gDNA using the BIO-X-ACT™ Long DNA Polymerase kit (Biolone, Taunton, MA) and the following primers complementary to exons 55 and 57: c.55F – 5'CTAGGAGAAAACGAAGGCAGAC3' and c.57R – 5'TCA-ACTGTCAGGTTTTGCATG3'. Resolved PCR fragments were purified using the MinElute PCR Purification Kit (Qiagen, Germantown, MD) and sequenced with the internal primer g.INT55F–5'CTCTACAAGCCAGCAATTCCAC3'. A rapid deletion-specific PCR was developed using the following primers: g.INT55-F2 – 5'ATCAGCTG-GAGAACAGAGAGGC3' and g.INT57-R – 5'-GTTTCAGTGGCTGATTCTTAGAGTTTC3'. Because this fragment only amplifies in deletion-positive individuals, it was multiplexed with an internal control (*DYSF* exon 20).

Results

Patients

Table 1 summarizes the clinical, neuroradiological and neuropathological data. Except for patients 11 and 20, all had symptoms since infancy. Patient 11 had a peculiar clinical picture with progressive spastic paraparesis followed later by a slowly progressive neuropathy. Pregnancy and delivery had been uneventful. Nerve conduction studies (at the age of 14 years) revealed delayed F waves. While myopathy was not clinically evident,

variation in fibre size was observed in the muscle biopsy. Patient 20 also had a milder phenotype with myopathy and clinically a mild neuropathy. MRI, performed in 22 patients, revealed white matter changes in all but one patient (tested in the neonatal period where these changes are not always visible). In another patient, this was observed in the computerized tomography scan. In three patients, cerebral changes included abnormal gyration. Seizures were reported only in the oldest patient. Twenty-two patients presented total absence of laminin- $\alpha 2$ staining in muscle, two patients presented partial absence and in a further two patients, this was not determined.

Identification of *LAMA2* gene mutations

Causative mutations were identified in all 26 patients (Table 2). Only one mutant allele was identified in patients 19 and 24; the mother of patient 24 carried the mutation, while the parents of patient 19 were not tested.

A total of 18 different mutations were identified, 14 of which have not been reported previously. Distribution by type was as follows: four nonsense mutations (22%), four duplications (22%), five deletions (28%), three splicing mutations (17%) and two missense mutations (11%). The two most frequent mutations were c.3085C>T (11 disease

Table 2. Mutations identified in the *LAMA2* gene

Patient	Mutations ^{a,b}	Gene location	Effect on mRNA	Predicted polypeptide change ^c	Reference
1 and 15	c.[7750-1713_7899-2153del]+[7750-1713_7899-2153del]	Introns 55–56	Frameshift	p.Ala2584HisfsX8	This report
2 and 10	c.[3976C>T]+[3976C>T]	Exon 27	PTC	p.Arg1326X	(13)
3, 13, and 18	c.[3085C>T]+[3085C>T]	Exon 22	PTC	p.Arg1029X	(13)
4	c.[4739dupG]+[7490_7493dupAAGA]	Exon 33 Exon 54	Frameshift Frameshift	p.Leu1581ProfsX5 p.Asp2498GlufsX4	This report This report
5	c.[1854_1861dupACGTGTTC]+[1854_1861dupACGTGTTC]	Exon 13	Frameshift	p.Leu621HisfsX7	(13)
6	c.[8244+1G>A]+[7750-1713_7899-2153del]	Intron 58 Intron 55–56	Splicing Frameshift	p.Pro2693ValfsX12 p.Ala2584HisfsX8	This report This report
7	c.[363C>A]+[7750-1713_7899-2153del]	Exon 3 Intron 55–56	PTC Frameshift	p.Tyr121X p.Ala2584HisfsX8	This report This report
8	c.[4318C>T]+[4739dupG]	Exon 30 Exon 33	PTC Frameshift	p.Gln1440X p.Leu1581ProfsX5	This report This report
9, 14, and 23	c.[3085C>T]+[7750-1713_7899-2153del]	Exon 22 Intron 55–56	PTC Frameshift	p.Arg1029X p.Ala2584HisfsX8	(13) This report
11	c.[1854_1861dupACGTGTTC]+[3832G>T]	Exon 13 Exon 26	Frameshift Missense	p.Leu621HisfsX7 p.Gly1278Cys	(13) This report
12	c.[1854_1861dupACGTGTTC]+[7750-1713_7899-2153del]	Exon 13 Intron 55–56	Frameshift Frameshift	p.Leu621HisfsX7 p.Ala2584HisfsX8	(13) This report
16	c.[3085C>T]+[5234+1G>A]	Exon 22 Intron 36	PTC Splicing	p.Arg1029X p.Val1765SerfsX21	(13) This report
17	c.[3976C>T]+[8776_8792del]	Exon 27 Exon 62	PTC Frameshift	p.Arg1326X p.Thr2926TrpfsX14	(13) This report
19	c.[1798_1800delGGA]+[=]	Exon 13 ?	Codon del ?	p.Gly600del ?	This report ?
20	c.[8613dupC]+[412T>C]	Exon 61 Exon 4	Frameshift Missense	p.Ser2872HisfsX34 p.Tyr138His	This report This report
21	c.[2049_2050delAG]+[6993-2A>C]	Exon 14 Intron 49	Frameshift Splicing?	p.Arg683SerfsX20 ?	(11) This report
22 and 25	c.[5234+1G>A]+[5234+1G>A]	Intron 36	Splicing	p.Val1765SerfsX21	This report
24	c.[3085C>T]+[=]	Exon 22 ?	PTC ?	p.Arg1029X ?	(13) ?
26	c.[8443_8450delACAGTTCA]+[8443_8450delACAGTTCA]	Exon 60	Frameshift	p.Thr2815AlafsX11	This report

cDNA, complementary DNA; del, deletion; PTC, premature termination codon.

^aMutations described according to Human Genome Variation Society nomenclature (23).

^bcDNA reference sequence with accession number NM_000426.3.

^cDeduced from the changes detected at the genomic or cDNA level.

alleles) and c.7750-1713_7899-2153del (10 disease alleles) together accounting for 42% of the mutant alleles (21/50).

The in-frame deletion c.1798_1800delGGA (p.Gly600del) and the missense variants c.412T>C (p.Tyr138His) and c.3832G>T (p.Gly1278Cys) were not detected in 300 normal alleles. Additionally, protein alignments indicated that the missense mutations affected highly conserved amino acids (Data S1, supporting information online). The single residue deletion (p.Gly600del), although coinciding with a known missense polymorphism in humans and between species (p.Gly600Arg), may have a detrimental effect on protein folding and/or function. The GENSCAN program predicted that the splice mutations c.5234+1G>A and c.8244+1G>A promote skipping of exons 36 and 58, respectively, whereas c.6993-2A>C should disrupt the acceptor splice site with subsequent use of a cryptic splice site located 7 bp into exon 50. Transcript analysis in patients 6 and 16 confirmed the *in silico* prediction for the former mutations, showing full skipping of the neighbouring preceding exons (data not shown). However, no muscle or fibroblast specimens from patient 21 were available for cDNA analysis to confirm the effect of c.6993-2A>C.

A total of 55 polymorphisms were also identified in the course of this study, 22 of which had not been reported previously (Data S2, supporting information online).

All the variants detected in this study were submitted to the *LAMA2* LSDB (<http://www.lovd.nl/LAMA2>).

Characterization of a new gross deletion

A large genomic deletion was first suspected in patient 1 when exon 56 failed to amplify. Further evidence was provided by haplotype analysis in patient 12, seen to be hemizygous for three informative SNPs in exon 56 (Fig. 1a). cDNA analysis in patients 1 and 12 revealed the presence of a smaller fragment missing the entire exon 56 (Fig. 1b). Southern blotting and hybridization indicated that the genomic deletion was approximately 5 kb long (Fig. 2a). This was confirmed by long-range PCR (Fig. 2b), which was used to further delineate the deletion endpoints. Sequencing revealed the absence of exon 56 plus a significant part of the flanking introns (4987 bp) characterized as c.7750-1713_7899-2153del (Fig. 2c). If translated, this frame shift predictably originates a truncated polypeptide (p.Ala2584HisfsX8).

The deletion-specific PCR that was developed enabled rapid screening of additional individuals. In all, 8 patients presented the c.7750-1713_7899-2153del mutation (2 homozygous and 6 heterozygous), and 10 family members were found to be carriers (data not shown).

Discussion

We describe the nature and frequency of *LAMA2* mutations in a group of 26 CMD patients. In this relatively large cohort, the high mutation detection rate of 96% (50/52 disease alleles) was achieved by direct gDNA sequencing or by combining this with RT-PCR analysis in cases where

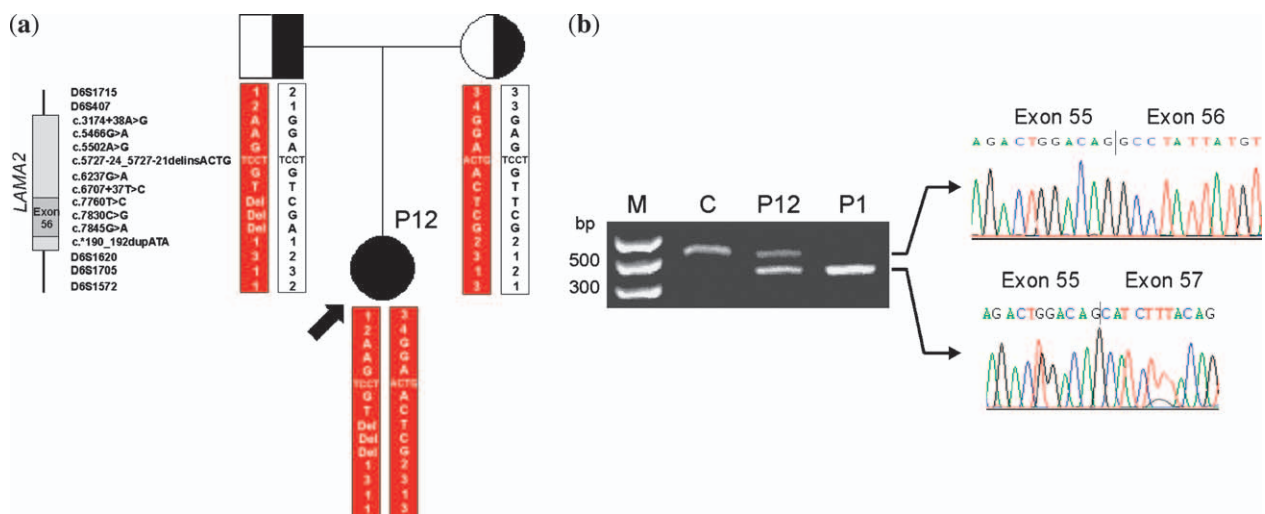


Fig. 1. (a) Pedigree of family/patient 12 showing analysis with microsatellite markers around the *LAMA2* locus and intragenic single nucleotide polymorphisms. Haplotyping suggested the presence of a heterozygous deletion of exon 56. (b) Complementary DNA analysis of *LAMA2* transcripts by Reverse transcription-polymerase chain reaction amplification of exons 54–58. Results revealed a smaller fragment missing the entire exon 56 in patients 1 and 12. C, control; M, molecular weight marker; P, patient.

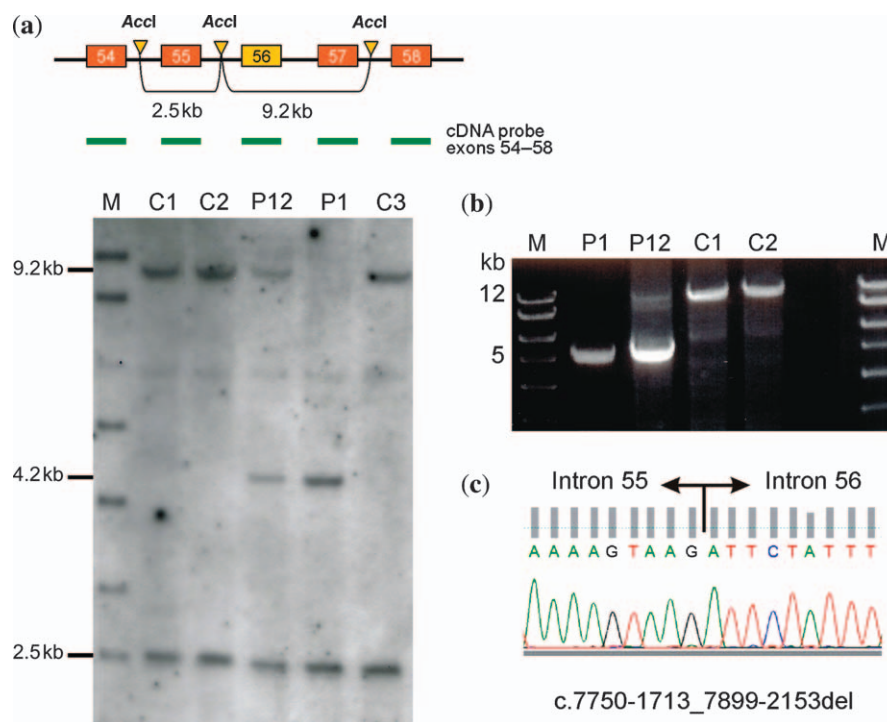


Fig. 2. (a) The size of the genomic deletion was determined by Southern blotting and hybridization using a complementary DNA probe encompassing exons 54–58. (b) Long-range PCR amplification of the genomic region between exons 55 and 56. (c) The resulting PCR fragments were sequenced revealing the deletion of exon 56 and part of the flanking intronic sequences (4987 bp). C, control; M, molecular weight marker; P, patient; PCR, polymerase chain reaction.

only one mutation had been detected. In patients 19 and 24, no adequate specimens were available for mRNA studies, and these were the only two cases where a single causal mutation was identified.

Similar previous studies, in a significant number of patients, have reported lower mutation detection rates ranging from about 60% to 80% (11, 12, 14). Because *LAMA2* is a large gene, most groups applied indirect mutation scanning techniques such as SSCA or denaturing high performance liquid chromatography combined with RT-PCR. However, these approaches may not detect all the mutations that are possibly present. Pegoraro et al. (11) also described the use of the protein truncation test (PTT) applied to *LAMA2* mutation detection. Because the majority of the mutations described in this gene cause premature translation termination, the use of this technique allowed the identification of 80% of the mutations in their cohort. Incomplete sensitivity was attributed to failure in the amplification of the mutated allele, the presence of mutations in the primer binding site or the fact that PTT is limited to the detection of nonsense mutations and mutations that alter the reading frame – small in-frame deletions/duplications or missense mutations – could be missed (11).

Almost 30% of the mutations described to date in the *LAMA2* gene are of the nonsense type. In our group of patients, these were also found to be the most frequent, comprising 18 of the 50 disease alleles (36%); however, c.3085C>T (p.Arg1029X) alone accounted for 11 of these.

A new 5-kb genomic deletion, encompassing exon 56, was also seen to be very frequent in this cohort (20% mutated alleles). To our knowledge, this is the first fully characterized genomic deletion described in *LAMA2*. A previous report documents an exon 56 deletion detected by RT-PCR (12); however, it remains uncertain that the genomic defect is the same. Although this mutation was detected in Portuguese and Spanish patients, no common haplotypes were found using STRs flanking the *LAMA2* locus (data not shown). These preliminary results point towards either a single ancient mutational event that has suffered genetic drift or a recurrent event that has occurred on different genetic backgrounds.

Quantitative assays such as multiple ligation-dependent probe amplification should improve mutation detection rates, enabling the identification of other gross rearrangements, which are probably underestimated in the *LAMA2* gene. Such changes could account for a reasonable number of MDC1A alleles and should therefore be

considered in the diagnostic setting, especially in patients with a single mutation identified by routine screening methods. An important implication in the failure to detect gross heterozygous deletions is that the identification of a single variant sequence may be erroneously interpreted as a homozygous mutation if masked by a coincident exonic deletion. Moreover, the absence of the variant in one of the parents may be mistaken as evidence of a *de novo* occurrence.

As expected, given the patients' inclusion criteria for the molecular study, the majority of mutations in our series were predictably truncating (94% of the mutated alleles). In line with previous observations (11), strict genotype/phenotype correlations were difficult to establish in such severely affected patients. Nevertheless, patients 11 and 20, who were compound heterozygous for a truncating and a missense mutation, presented partial deficiency of laminin- α 2 in muscle. The clinical phenotype of these patients was also clearly milder with a later age of onset (2–3 years) when compared with the other patients (birth to 4 months). They achieved the ability to walk unsupported, which is rarely observed in MDC1A. Patient 19 has also kept independent ambulation till the age of 17 years; in this case, a single in-frame deletion was detected, but laminin- α 2 expression in muscle has not been accessed. These patients may belong to a clinical sub-form of CMD with primary partial laminin- α 2 deficiency, as has been suggested previously (18).

Our results have enabled the reliable determination of carrier status in additional family members and a more accurate prenatal diagnosis in several pregnancies. The importance of extensive molecular characterization is increasingly recognized also in light of the new therapeutic strategies that are currently being developed, such as the use of antisense oligonucleotides (19) and drug-induced stop codon read-through in MDC1A patients (20). To this end, mutations need to be contextualized. In those inducing premature termination codons, the extent of nonsense-mediated mRNA decay needs to be assessed by real-time RT-PCR (20, 21, 22) because mRNA stability influences the efficacy of transcript rescue. It is thus foreseeable that future demand on diagnosis will include qualitative and quantitative transcript analysis to identify the cases amenable to the different targeted therapies.

Supporting information

Data S1. Partial laminin- α 2 protein sequences alignment.

Data S2. New polymorphisms detected in *LAMA2*.

Supporting information are available as part of the online article at <http://www.blackwell-synergy.com>

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