

Current understanding and treatment of cardiac and skeletal muscle pathology in laminin- α 2 chain-deficient congenital muscular dystrophy

This article was published in the following Dove Press journal:
The Application of Clinical Genetics

Quynh Nguyen^{1,*}
Kenji Rowel Q Lim^{1,*}
Toshifumi Yokota^{1,2}

¹Department of Medical Genetics, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada; ²The Friends of Garrett Cumming Research & Muscular Dystrophy Canada, HM Toupin Neurological Science Research Chair, Edmonton, AB, Canada

*These authors contributed equally to this work

Abstract: Congenital muscular dystrophy (CMD) is a class of severe early-onset muscular dystrophies affecting skeletal/cardiac muscles as well as the central nervous system (CNS). Laminin- α 2 chain-deficient congenital muscular dystrophy (LAMA2 MD), also known as merosin-deficient congenital muscular dystrophy type 1A (MDC1A), is an autosomal recessive CMD characterized by severe muscle weakness and degeneration apparent at birth or in the first 6 months of life. LAMA2 MD is the most common congenital muscular dystrophy, affecting approximately 4 in 500,000 children. The most common cause of death in early-onset LAMA2 MD is respiratory tract infection, with 30% of them dying within the first decade of life. LAMA2 MD is caused by loss-of-function mutations in the *LAMA2* gene encoding for the laminin- α 2 chain, one of the subunits of laminin-211. Laminin-211 is an extracellular matrix protein that functions to stabilize the basement membrane and muscle fibers during contraction. Since laminin- α 2 is expressed in many tissue types including skeletal muscle, cardiac muscle, Schwann cells, and trophoblasts, patients with LAMA2 MD experience a multi-systemic clinical presentation depending on the extent of laminin- α 2 chain deficiency. Cardiac manifestations are typically associated with a complete absence of laminin- α 2; however, recent case reports highlight cardiac involvement in partial laminin- α 2 chain deficiency. Laminin-211 is also expressed in the brain, and many patients have abnormalities on brain imaging; however, mental retardation and/or seizures are rarely seen. Currently, there is no cure for LAMA2 MD, but various therapies are being investigated in an effort to lessen the severity of LAMA2 MD. For example, antisense oligonucleotide-mediated exon skipping and CRISPR-Cas9 genome editing have efficiently restored the laminin- α 2 chain in mouse models in vivo. This review consolidates information on the clinical presentation, genetic basis, pathology, and current treatment approaches for LAMA2 MD.

Keywords: LAMA2, exon skipping, genome editing, non-homologous end joining, phosphorodiamidate morpholino oligomer, CRISPR/Cas9

Introduction

Laminin- α 2 chain-deficient muscular dystrophy (LAMA2 MD), or merosin-deficient congenital muscular dystrophy type 1A (MDC1A), is an autosomal recessive disorder caused by *LAMA2* gene mutations that lead to loss of laminin- α 2.¹ The extent of laminin- α 2 deficiency dictates disease severity in most cases. Complete laminin- α 2 loss results in an early-onset, congenital form of LAMA2 MD characterized by severe hypotonia, muscle weakness, skeletal deformity, non-ambulation, and respiratory insufficiency.^{2,3} On the other hand, partial loss of laminin- α 2 manifests as a late-onset, limb girdle-type muscular dystrophy form of LAMA2

Correspondence: Toshifumi Yokota
Faculty of Medicine and Dentistry,
University of Alberta, 8-31 Medical
Sciences Building, 8613-114 St,
Edmonton, AB T6G2H7, Canada
Tel +1 780 492 1102
Fax +1 780 492 1998
Email toshifum@ualberta.ca

MD. This presents with similar symptoms as early-onset LAMA2 MD albeit considerably milder and with wider phenotypic variability; most patients develop the ability to walk.^{1,2} Cardiac disease is associated with either case. There is currently no cure for LAMA2 MD.

The global prevalence of LAMA2 MD is poorly known and varies across sources. Based on available estimates, it affects about 1–9/1,000,000 individuals.² Early-onset LAMA2 MD is the most common form of congenital muscular dystrophy (CMD) globally, affecting about 30% of the CMD patients in Europe and 6% of the patients in Japan.^{4–6} One study in Denmark revealed that late-onset LAMA2 MD accounts for 2.3% of the limb girdle-type muscular dystrophy cases.⁷

In 1994, Tomé et al first described LAMA2 MD as a form of CMD characterized by loss of laminin-211, then called merosin.⁸ The following year, Helbling-Leclerc et al determined this was caused by *LAMA2* gene mutations.⁹ Laminin- $\alpha 2$, interacting with laminin- $\beta 1$ and - $\gamma 1$, forms the cruciform-like laminin-211 structure.² There are numerous laminin isoforms, formed from various combinations of α , β , and γ chains, but laminin-211 is the major one in the neuromuscular system.¹⁰ Laminins link cells to the basement membrane via binding to cell surface receptors and also stabilize the basement membrane through interactions with each other or with extracellular matrix (ECM) proteins.² Laminin- $\alpha 2$ deficiency results in a corresponding loss of laminin-211 and the disruption/absence of the basement membrane surrounding muscle fibers. While the specific molecular mechanisms are an area of active research, this ultimately leads to the observed pathology in LAMA2 MD.

Since no curative treatments are available for LAMA2 MD, current strategies in the clinic are focused on management. This usually takes the form of, among others, feeding supplementation for difficulties eating and swallowing, non-invasive ventilation support for respiratory insufficiency, and physical therapy for joint contractures, spinal defects, and other issues.¹ As these only provide temporary relief, it is encouraging that many groups are currently developing therapies for LAMA2 MD. Different approaches have been devised with varying rates of success, ranging from laminin- $\alpha 2$ replacement to the modulation of cellular events downstream of laminin- $\alpha 2$ loss such as apoptosis and fibrosis. Strategies to correct the defective *LAMA2* gene by genome editing or pre-mRNA using anti-sense oligonucleotides have also been tested in mouse models and seem promising.

In this review, we provide a comprehensive overview of LAMA2 MD, its clinical presentation, pathophysiology, as well as the approaches that have been developed to treat it.

Clinical presentation

Skeletal muscle-related features

The clinical manifestations of LAMA2 MD vary depending on the degree of laminin- $\alpha 2$ deficiency. Complete absence of laminin- $\alpha 2$ presents as severe early-onset CMD, while partial laminin- $\alpha 2$ deficiency often leads to mild late-onset, limb girdle-type muscular dystrophy.¹ Children with severe LAMA2 MD present with a weak cry, generalized muscle weakness and profound muscle hypotonia at birth.^{11,12} Most of these children have delayed motor developmental milestones and very few acquire independent ambulation.³ With assistance, a small percentage of LAMA2 MD patients may be able to walk, but they invariably lose the ability later on in life. As the disease progresses, affected individuals can develop facial muscle weakness and macroglossia, which result in typical myopathic facies with protruded tongue.¹

Early-onset LAMA2 MD is also characterized by respiratory involvement. Weakness of intercostal and accessory muscles results in progressive restriction of the chest wall, decreased lung volume, reduced alveolar gas exchange, and eventually restrictive respiratory insufficiency. Affected individuals also experience skeletal changes such as proximal joint contractures and scoliosis.¹³ During the early years, contractures tend to occur in the shoulder, elbow, hip, and knee, and progress distally. Within the first decade of life, scoliosis may result in lumbar and thoracic lordosis, which interferes with breathing.¹⁴ As a consequence, most children with severe LAMA2 MD require ventilatory support at various points in their life.³ Recurrent chest infections due to reduced secretion clearance are another common presenting feature. Respiratory tract infection is the most common cause of death in early-onset LAMA2 MD children, with 30% of them dying within the first decade of life.¹⁵

Complete absence of laminin- $\alpha 2$ often manifests as failure to thrive in children.¹⁶ Feeding difficulties, swallowing abnormalities, and difficulty in chewing all contribute to poor weight gain in affected children.¹⁶ On top of that, recurrent infections further exacerbate the problem. Most children with early-onset LAMA2 MD fall below the third percentile for weight, and some require enteral feeding to meet their nutritional requirement.³

Since laminin- $\alpha 2$ is distributed widely in the body, including the brain, central nervous system involvement in LAMA2 MD is inevitable.¹⁷ White matter abnormalities, often presenting as white matter hyperintensities on cerebral MRI, can be observed in patients at 6 months of age. This manifestation is most helpful for diagnostic purposes since it is not associated with any functional impairment.¹ Structural brain changes such as bilateral occipital pachygyria or dysplastic cortical changes were reported in a small percentage of affected children and were associated with intellectual disability or epilepsy.^{18,19} Progressive sensorimotor neuropathy due to myelination defects in the peripheral nervous system was also reported; however, these findings are usually mild and not clinically significant.²⁰

Individuals with partial laminin- $\alpha 2$ deficiency have milder disease manifestations, later onset of symptoms and are typically classified as having limb girdle-type muscular dystrophy.^{1,2} Affected people usually stay asymptomatic during the first few years of life, although early muscle degeneration may manifest as a delay in walking or as proximal muscle weakness. Patients may also present with elevated creatine kinase (CK) levels, typical dystrophic muscle features, respiratory insufficiency, and abnormal brain MRI.

Cardiac features

Laminin- $\alpha 2$ chain expression is particularly high in the heart.²¹ However, cardiac involvement has historically not been the focus of LAMA2 MD clinical presentation.^{1,2} There were only a few studies reporting cardiac manifestations in patients with LAMA2 MD and none of these were comprehensive. Lately there has been more evidence of cardiac involvement in LAMA2 MD, which raises the question of whether cardiac involvement is truly not a major complication of the disorder or is simply under-reported in the literature.^{22,23} Similar to other muscular dystrophies, with improved ventilatory support and respiratory management, cardiac manifestations may become more important and require more attention in the treatment and management of LAMA2 MD patients.

Cardiac abnormalities are predominantly reported in patients with complete laminin- $\alpha 2$ deficiency. To the best of our knowledge, only two cases of partial laminin- $\alpha 2$ deficiency patients presenting with cardiac involvement have been reported.^{22,23} One study specifically investigated cardiac involvement in 16 children with CMD using two-dimensional echocardiography.²⁴ Two of 6

children with LAMA2 MD had significant left ventricular dysfunction with ejection fractions (EFs) of less than 40%. Both of these children had complete laminin- $\alpha 2$ deficiency. The average EF of children with complete laminin- $\alpha 2$ deficiency was 43%, which was significantly lower than that of the partial deficiency group at 53%.

Another bibliographical review looked at 248 published patient cases with abnormal immunohistochemical staining of laminin- $\alpha 2$.¹¹ Cardiac features were described in 20 cases, of which 7 had clinically relevant cardiac involvement. Cardiac abnormalities manifested as either a right bundle branch block, dilated cardiomyopathy or borderline changes in cardiac function. In another LAMA2 MD study, cardiac phenotypes were evaluated in 15 out of 51 patients.³ Five patients with a complete absence of laminin- $\alpha 2$ had cardiac abnormalities that include mitral valve regurgitation, pulmonary hypertension, palpitations, and wall motion hypokinesia as seen on the echocardiogram. Normal echocardiograms were observed in 7 cases with complete laminin- $\alpha 2$ deficiency and 3 cases with residual laminin- $\alpha 2$ expression. Documentation on the cardiac status of the remaining patients was not available.

The first case of a partial laminin- $\alpha 2$ defect presenting with cardiac involvement was documented in a patient with two different mutations in the *LAMA2* gene.²³ At 30 years of age, the first symptoms reported for this patient were palpitations and precordial pain. He also had a single episode of syncope. However, clinical evaluation did not show any signs of cardiomyopathy at the time. Electrocardiography (ECG) showed sinus rhythm, but sporadic ventricular ectopic beats were detected by 24-hr Holter ECG monitoring. Mild left ventricular dilation and reduced EF were observed on the echocardiogram. EF was confirmed to be about 39% by angiocardiography. His cardiac status remained unchanged until age 40 when he experienced an episode of syncopal ventricular tachycardia. Long-term evaluation led to a diagnosis of dilated cardiomyopathy with a progressive decrease in ventricular function (EF of 33%), which required implantation of an intracardiac defibrillator.

The second case was also characterized as having partial laminin- $\alpha 2$ deficiency with severe cardiac involvement.²² Echocardiography showed impaired left ventricle contractility and mitral valve prolapse. Cardiac function progressively declined with left ventricle dysfunction and dilatation (fractional shortening of 18%). The patient was diagnosed with dilated cardiomyopathy and, eventually, congestive heart failure NYHA class II-III.

Cardiac abnormalities were previously thought to only manifest in severe early-onset LAMA2 MD with complete absence of laminin- α 2.^{1,2} However, from the above-mentioned studies, we want to emphasize the need for routine cardiac assessment in patients with LAMA2 MD due to the potential for severe presentation even in those with residual expression of the laminin- α 2 chain.

Diagnosis

In order to establish or confirm a diagnosis of LAMA2 MD, various strategies can be used including history taking, physical examination, laboratory testing, diagnostic imaging, and molecular genetic testing. For early-onset LAMA2 MD, clinical features indicative of muscle weakness and degeneration such as motor delay, muscle weakness, and profound hypotonia can be the first signs that clinicians notice upon physical examination.^{1,25} Growth measurement and monitoring in children are essential in the diagnosis and intervention of LAMA2 MD, since children with complete absence of laminin- α 2 typically present with failure to thrive.¹⁶ As mentioned earlier, serum CK levels are usually elevated in patients with LAMA2 MD. Although serum CK is not a specific marker, it is still useful in the diagnosis and confirmation of LAMA2 MD. Expression levels of laminin- α 2 can be evaluated using immunohistochemistry of skin or muscle biopsies. Antibodies against various regions of the laminin- α 2 chain can be used to detect the presence of the protein as well as its level of expression. Histology studies using hematoxylin and eosin staining of muscle may show characteristic findings of muscle degeneration such as increased numbers of centrally nucleated fibers, muscle fibrosis, and fat infiltration.¹ Structural brain alterations and white matter abnormalities in LAMA2 MD patients can also be revealed by brain MRI.^{1,2,18} Typical cardiovascular diagnostic tools such as ECG and echocardiography are useful in the evaluation of cardiac abnormalities, especially in patients with a complete absence of laminin- α 2.^{1,21} Since *LAMA2* is the only gene directly affected in LAMA2 MD, molecular genetic testing is the most definitive approach to confirm patient diagnosis.

It is essential to distinguish LAMA2 MD from other neuromuscular disorders since the clinical features and laboratory findings for LAMA2 MD can be non-specific. Early-onset LAMA2 MD needs to be differentiated from other forms of CMD, congenital myopathies and spinal muscular atrophy (SMA). Other neuromuscular disorders are not typically associated with lack of laminin- α 2

staining in immunohistochemistry or white matter changes on the brain MRI.¹ Histological studies prove to be most useful in distinguishing LAMA2 MD from congenital myopathies, since the latter often show pathognomonic structural abnormalities that are indicative of each condition.¹ With regard to SMA as a differential diagnosis, SMA typically presents with rapid motor impairment and tongue fasciculations. A denervation-reinnervation profile from muscle biopsy findings or electromyography is suggestive of a diagnosis of SMA. Late-onset LAMA2 MD needs to be differentiated from other forms of limb girdle-type muscular dystrophy.¹ Despite the overlap in clinical presentation among these diseases, protein and genetic studies can help provide a more definitive diagnosis of LAMA2 MD.

Pathophysiology

Laminin- α 2 chain biology

LAMA2 MD is caused by a complete or partial deficiency of laminin- α 2 chain protein. Laminin- α 2 chain is encoded by the *LAMA2* gene, which is transcribed and translated into a 390-kDa protein.^{26,27} After translation, the laminin- α 2 chain is cleaved at the 2580th amino acid into a 300-kDa N-terminal fragment and an 80-kDa C terminal fragment that are non-covalently associated with each other.^{28,29} Laminin- α 2 is a component of a heterotrimeric, cross-shaped molecule known as laminin-211 (or merosin).^{26,30,31} There are many laminin isomers with different compositions and arrangements of laminin subunits. Laminin-211 is the major isoform expressed in the basement membrane of cardiac and skeletal muscle.¹⁰ Laminin-211 is also found in Schwann cells and trophoblasts.¹⁰ Laminin-211 is an essential part of the dystrophin-glycoprotein complex (DGC), which provides mechanical support and stabilizes muscle cell membranes during contraction and relaxation cycles (Figure 1a).^{32,33} Besides laminin- α 2, laminin-211 is composed of 2 other subunits: laminin- β 1 and laminin- γ 1. Once laminin- α 2 is translated, it joins laminin- β 1 and laminin- γ 1 to form laminin-211.³⁴ How laminin-211 is delivered to the muscle cell or how this process of delivery is regulated is not well understood. It has been demonstrated that the N-terminal domain of laminin- α 2 is essential for laminin-211 self-assembly at the muscle cell surface.^{2,35} Laminin-211 networks are associated with cell surface receptors, collagen IV network, and heparan sulfate proteoglycans.^{2,36} The C-terminus of laminin- α 2, which contains 5 laminin G (LG)-like domains (LG1-5), is also important for linking

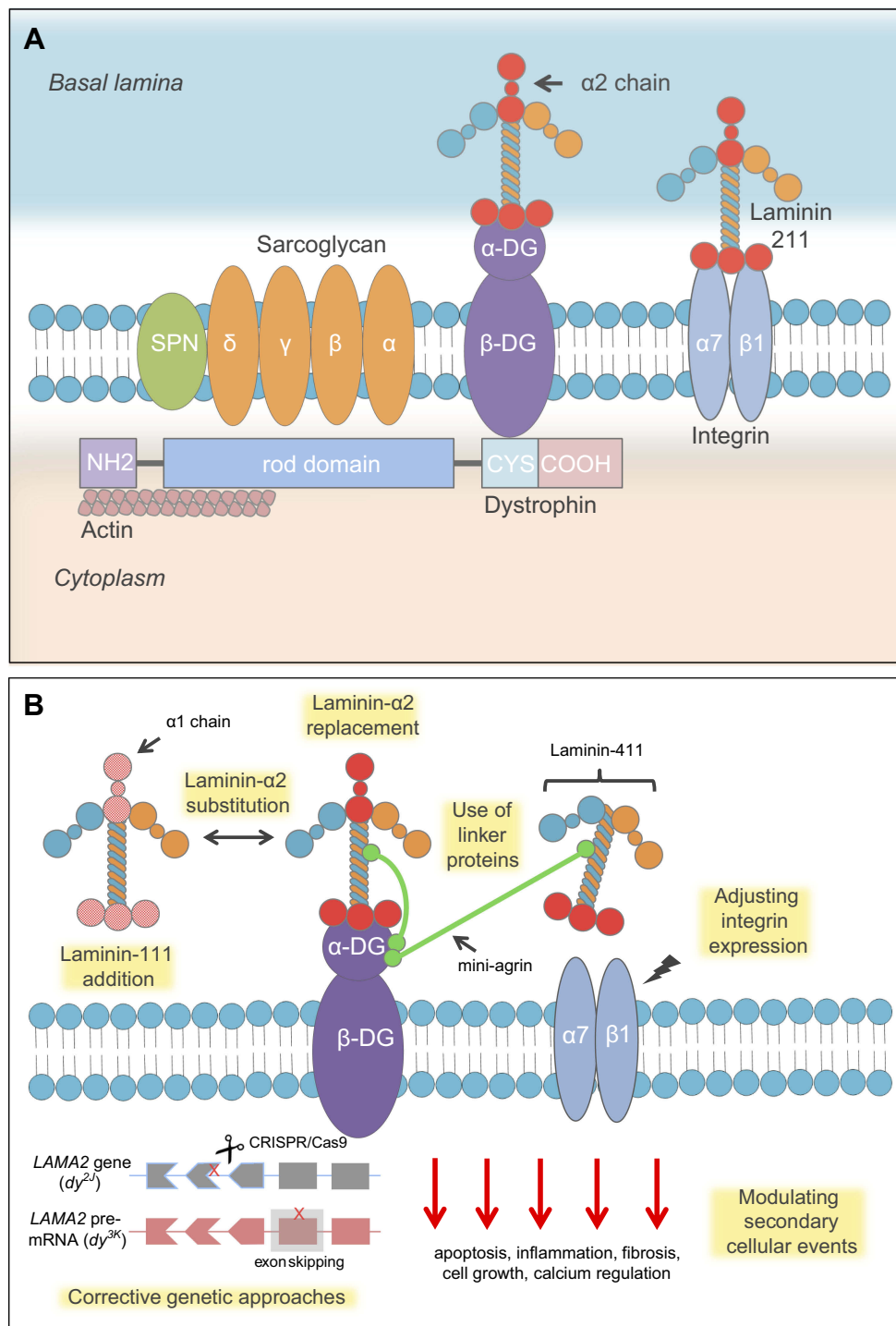


Figure 1 (A) Laminin- $\alpha 2$ and the dystrophin glycoprotein complex. Laminin- $\alpha 2$ interacts with the laminin β and γ chains to form laminin-211, which binds both α -dystroglycan (α -DG) and the $\alpha 7 \beta 1$ integrin. Other members of the dystrophin glycoprotein complex are also depicted, with the dystrophin domains shown. β -DG, β -dystroglycan; SPN: sarcospan. **(B)** Therapeutic strategies developed for LAMA2 MD. An overview of the various LAMA2 MD treatments (in yellow boxes) is shown. For the LAMA2 gene and pre-mRNA diagrams depicted, a red "X" represents the location of the indicated mouse model mutation.

Abbreviations: CMD, congenital muscular dystrophy; CNS, central nervous system; LAMA2 MD, Laminin- $\alpha 2$ chain-deficient muscular dystrophy; MDC1A, merosin-deficient congenital muscular dystrophy type 1A; NHEJ, nonhomologous end-joining; PMO, phosphorodiamidate morpholino oligomer; ECM, extracellular matrix; CK, creatine kinase; ECG, electrocardiography; EF, ejection fraction; SMA, spinal muscular atrophy; DGC, dystrophin-glycoprotein complex; LG, laminin G; CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats; TA, tibialis anterior; $\alpha 2$ LN, laminin- $\alpha 2$ N-terminal domain; MCK, muscle creatine kinase; CNF centrally nucleated fiber; EHS, Engelbreth-Holm-Swarm; IGF-I, insulin-like growth factor I; EDL, extensor digitorum longus; SOL, soleus; MLC, myosin light chain; TGF- $\beta 1$, transforming growth factor $\beta 1$; AO, antisense oligonucleotide; DMD, Duchenne muscular dystrophy.

laminin-211 to the cytoskeleton in skeletal muscle cells via dystroglycan and integrin $\alpha 7 \beta 1$.^{37,38} Dystroglycan has 2 subunits: an α -dystroglycan subunit which binds laminin-211 at the LG4-5 and LG1-3 domains, and a β -dystroglycan subunit which binds dystrophin, a major protein linking the actin cytoskeleton of muscle cells to the DGC and, thus, the ECM.^{33,39–41} Integrin-laminin-211 association requires the LG1-3 domains of the laminin- $\beta 1$, laminin- $\gamma 1$, and laminin- $\alpha 2$ chains.^{2,42–45}

Genetics of LAMA2 MD

Laminin- $\alpha 2$ is encoded by the *LAMA2* gene, which maps to chromosome 6q22.33 and is composed of 65 exons.^{26,27,46} Pathogenic variants in the *LAMA2* gene give rise to a group of muscular diseases collectively referred to as LAMA2 MD. LAMA2 MD is inherited in an autosomal recessive fashion. As mentioned in the introduction, the prevalence of LAMA2 MD varies significantly depending on the source. LAMA2 MD has a wide mutational spectrum, ranging from changes in the coding sequence that create premature stop codons, to splice site mutations that result in the translation of pathogenic protein isoforms. In fact, the most common reported variants are those that create premature stop codons, leading to truncation of the protein.³ Loss-of-function mutations in both copies of the *LAMA2* gene give rise to the more severe early-onset LAMA2 MD. These mutations were found scattered throughout the *LAMA2* coding region, with 55% clustering in exons 14, 25, 26, and 27.^{1,3} On the other hand, missense variants, in-frame deletions and splice site mutations are often associated with late-onset LAMA2 MD where residual laminin- $\alpha 2$ chain expression can still be detected. It was estimated that 18.4% of the disease-causing variants in LAMA2 MD is due to large deletions and duplications.^{47,48}

For the most part, mutations that result in complete laminin- $\alpha 2$ deficiency lead to a more severe phenotype. In the study of 51 patients with confirmed LAMA2 MD that we have described earlier, those with a complete deficiency of laminin- $\alpha 2$ showed earlier symptom onset (at or within 7 days of birth), and were more likely to never achieve independent ambulation and to require ventilatory and enteral feeding support.³ In another study of 26 LAMA2 MD patients, only 3 were able to achieve independent walking, 2 of whom harbored a missense or a single in-frame deletion mutation in one allele of the *LAMA2* gene in heterozygosity with a frame-shifting mutation.⁴⁹ All patients with frame-shifting mutations in both copies of the gene were unable to acquire independent ambulation. However, there are exceptions to this

rule. Geranmayeh et al³ reported two individuals with complete laminin- $\alpha 2$ deficiency, both of whom had generally milder phenotypes, gained independent ambulation, and did not require feeding or ventilatory support.³ In-frame deletions affecting the C-terminal region of the laminin- $\alpha 2$ chain, which is essential for linking laminin-211 to the cytoskeleton in muscle cells, result in severe phenotypes despite the detection of residual laminin- $\alpha 2$.²⁵ LAMA2 MD also shows intrafamilial clinical variability. Affected siblings with the same genotype may have different clinical manifestations.¹ As previously mentioned, LAMA2 MD patients may present with brain abnormalities that are associated with intellectual disability and seizures. However, no association was found between patient genotypes and the manifestation of nervous system disease phenotypes.^{1,47}

Pathogenesis

The primary mechanism for LAMA2 MD pathogenesis is a complete or partial deficiency of laminin- $\alpha 2$ in muscle. When the laminin- $\alpha 2$ chain is defective or absent, muscle fibers experience mechanical stress and become susceptible to tearing and fragmentation, resulting in tissue injury and degeneration. Following injury, infiltrating inflammatory cells and muscle stem cells (called satellite cells) coordinate their activities to restore tissue homeostasis.⁵⁰ However, in situations with chronic tissue damage such as in LAMA2 MD, inflammatory cell infiltration and fibroblast activation persist while satellite cells are being constantly depleted due to the muscle experiencing continuous cycles of degeneration and regeneration. Eventually, the muscle tissue is deposited with excessive amounts of ECM components and is replaced by permanent scars or fibrotic tissue.^{51–53} Transcriptomic and proteomic studies have indicated that the most upregulated genes in LAMA2 MD encode for ECM proteins and specific isoforms of proteins that are transiently expressed during normal muscle development and regeneration.^{54–56}

In LAMA2 MD, dystroglycan and integrin $\alpha 7 \beta 1$ expression levels are also altered. α -dystroglycan is reduced, while both glycosylated α -dystroglycan and β -dystroglycan levels are slightly increased.^{45,57–59} Although integrin $\alpha 7 \beta 1$ expression levels are increased, its assembly process at the muscle cell membrane is compromised.^{45,57,59,60} Integrin $\alpha 7 \beta 1$ is essential for satellite cell activation, which functions in muscle repair and regeneration.⁶¹ Reduction in integrin $\alpha 7 \beta 1$ activity and, subsequently, satellite cell function invariably result in

impaired muscle regeneration. Additionally, integrin $\alpha 7 \beta 1$ plays an important role in the survival of muscle fibers.⁴⁵ Integrin $\alpha 7 \beta 1$ dysregulation along with pathological alterations in other signaling pathways contribute to the abnormal skeletal muscle cell apoptosis observed in LAMA2 MD.²

Besides impaired regeneration, the imbalance between protein synthesis and protein breakdown is another factor leading to loss of muscle mass and muscle atrophy in LAMA2 MD. The ubiquitin-proteasome system and the autophagy-lysosome pathway, both of which function in protein degradation, are upregulated in LAMA2 MD.² There is also evidence of integrin $\alpha 7$ subunit involvement in the negative regulation of these pathways.⁶² Decreases in integrin $\alpha 7$ expression, therefore, can lead to over-activation of proteasome and autophagy activity in muscle cells.⁶³

Treatment strategies for LAMA2 MD

Therapeutic strategies for LAMA2 MD can be broadly classified into three types. The first aims to restore the structure and function of the basement membrane, as well as its interactions with adjacent cells. The second aims to modulate cellular events caused by laminin- $\alpha 2$ loss. Finally, the last group targets the genetic defect in LAMA2 MD, either through affecting mRNA processing or correcting the causative mutation using the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 system. We provide a summary of these approaches in Figure 1b and Table 1.

Treating the basement membrane

Laminin- $\alpha 2$ replacement and substitution

The most straightforward way to treat LAMA2 MD is to replenish what is lost. Using the *dy/dy* LAMA2 MD mouse model, Vilquin et al first demonstrated partial laminin- $\alpha 2$ replacement by primary muscle cell culture transplantation in 1996.⁶⁴ *dy/dy* mice carry a spontaneous mutation that results in very low to absent laminin- $\alpha 2$ expression in striated muscle basement membranes.^{65,66} Although *Lama2* has been mapped to the same region as the *dy* locus, the exact nature and location of *dy* remain unknown.⁶⁵ *dy/dy* mice exhibit progressive ataxia and muscle wasting. Histology reveals extensive fibrosis and generally smaller, fewer muscle fibers. These mice have decreased survival, with most dying by 6 months of age.⁶⁷ Allogeneic transplantation of primary myoblasts from

healthy mice to the tibialis anterior (TA) muscles of *dy/dy* mice resulted in up to 15.9% laminin- $\alpha 2$ -positive fibers on average, with younger recipients showing more laminin- $\alpha 2$ rescue.⁶⁴ Use of notexin and γ -irradiation increased the number to 27.8% on average. Syngeneic transplantation resulted in a mean 41.2% of laminin- $\alpha 2$ -positive fibers, while transplantation of immortalized myoblasts or a fibroblast cell line yielded little to none. In a separate study, the group showed that transplantation of pure myoblasts was also successful in producing laminin- $\alpha 2$ -positive fibers.⁶⁸ Since no other assessments were done, the functional benefit of the approach cannot be determined.

Evaluation of the benefits of transgenic *LAMA2* overexpression for LAMA2 MD treatment was reported by Kuang et al (1998).⁶⁹ Instead of *dy/dy*, they used the milder *dy^{2J}/dy^{2J}* and the more severe *dy^W/dy^W* mouse models. The *dy^{2J}/dy^{2J}*, *dy^W/dy^W*, and *dy^{3K}/dy^{3K}* (to be described later) mouse models have entirely different mutations from *dy/dy* mice; however, the *dy* nomenclature was retained for ease of classification. The *dy^{2J}/dy^{2J}* model has a spontaneous G-to-A donor splice site mutation in intron 2 of the *Lama2* gene.⁷⁰ This excludes exon 2 from the pre-mRNA and creates an in-frame deletion of the laminin- $\alpha 2$ N-terminal domain ($\alpha 2$ LN) responsible for polymerization. Laminin- $\alpha 2$ mRNA and protein expression are only slightly reduced in *dy^{2J}/dy^{2J}* mice, contributing to the decreased severity of phenotypes observed in this model. In contrast, the mutation in *dy^W/dy^W* mice was generated by targeted disruption of the *Lama2* gene, which led to severely reduced laminin- $\alpha 2$ expression.⁶⁹ *dy^W/dy^W* mice, therefore, have a worsened dystrophic phenotype, with most dying 2–4 weeks after birth or, in more recent reports, a median survival of ~8–14 weeks.⁷¹

Homozygous *dy^{2J}* and *dy^W* mice that were heterozygous for the human *LAMA2* transgene were examined.⁶⁹ The transgene was controlled by the muscle creatine kinase (MCK) promoter, which specifically expresses it in striated muscles. Transgenic *LAMA2* improved the overall phenotype of *dy^W/dy^W* mice, significantly increasing body weight and prolonging survival to at least 8 months. Less improvement was observed for transgenic *dy^{2J}/dy^{2J}* mice, due to their already mild phenotype. Histologically, both transgenic *dy^W/dy^W* and *dy^{2J}/dy^{2J}* mice had skeletal muscles that appeared nearly wild-type, with evidence of only a mild myopathy due to the appearance of a few centrally nucleated fibers (CNFs). This corresponded to a significant reduction in serum CK activity in these mice, to at least 50% of the non-transgenic levels.

Table 1 Summary of LAMA2 MD therapeutic strategies discussed in the review

Purpose	Strategy	Method/drug	Comments; challenges	Main refs
Basement membrane treatment	Laminin- α 2 replacement and substitution	Laminin- α 2 replacement	Cell therapy a potential approach, transgenic mice show disease amelioration; laminin- α 2 immunogenicity could be an issue	64,69
		Laminin- α 1 provision	Functionally compensates for laminin- α 2; large cDNA size poses delivery issues, laminin- α 1 is not exactly laminin- α 2	74,76
		Laminin-III therapy	Reduces dystrophy in LAMA2 MD mice, laminin-III can be derived from EHS tumors; pharmacokinetic study recommended	82,83
	Use of linker proteins	Mini-agrin	Enhances laminin association to α -dystroglycan; very focused, may need to be used with other linker proteins or therapies	86
	Adjusting integrin expression	α LNND	Enhances laminin polymerization and binding to collagen IV; same challenge as for mini-agrin	93,95
Modulating cellular events caused by laminin- α 2 loss		α 7 integrin overexpression	Transgenic LAMA2 MD mice had moderately improved lifespan, muscle function; other integrin targets can be explored	96
		β 1 integrin inhibition	RGD inhibition of β 1 integrin activity improved ECM composition, myofiber stability; more research into the role of α 7 β 1 in disease	97
	Enhancing cell growth	IGF-1, clenbuterol	Improved myofiber size, generally partial improvements on health and survival; treatment dose, administration, regimen needs work	100,103
	Reducing apoptosis	Bax inhibition, Bcl2 expression	Disease amelioration was successfully observed in transgenic mice; need to find a way to alter expression pharmacologically	104
	Inhibiting the immune response and fibrosis	Omigapil, doxycycline	Recently completed phase I clinical trial for omigapil in LAMA2 MD therapy; efficacy and other outcomes to be released	106,108,109
		Losartan, TXA127	TXA127 granted Orphan Drug status for treating LAMA2 MD; exact information on safety and efficacy remains to be seen	113,118
		Prednisolone, halofuginone, GTA, FTS, C3, galectin-3, osteopontin	Various effects on LAMA2 MD disease, from helpful to harmful; each needs in-depth study, side effects have to be considered	122-126
	Targeting other intra-cellular systems of regulation	Proteasome inhibition (MG-132, bortezomib)	Partially useful to having no effect at all in ameliorating LAMA2 MD; rethinking of the approach or search for more targeted inhibitors recommended	127-129
		Autophagy inhibition (3-MA)	Partially useful in ameliorating LAMA2 MD; same challenge as for proteasome inhibition	130
		Reducing calcium levels and its effects (cal-decrin, cyclophilin D inactivation)	Promising results for LAMA2 MD treatment; however, studies are very preliminary and more research into other outcomes of treatment is required	131,132
	Targeting metabolism (metformin)		Gender-specific therapeutic effect observed; mechanism of action largely unknown	133
	Targeting glycosylation (CT GalNAc transferase overexpression)		Ameliorates LAMA2 MD in dystrophic mice; mechanism of action also unclear, but may be linked to enhancing agrin expression	134

(Continued)

Table 1 (Continued).

Purpose	Strategy	Method/drug	Comments; challenges	Main refs
Genetic correction	Exon skipping	Lama2 exon 4 skipping PMO	Laminin- $\alpha 2$ protein production was restored in the dy^{3K} model, and dystrophic symptoms were treated; PMO delivery can be improved, patient applicability currently limited	138
	CRISPR/Cas9	Exon 2 inclusion	Lama2 mutation corrected in the dy^{2J} model, laminin- $\alpha 2$ production rescued, treated animals significantly improved; off-target effects are a concern, need strategies for other mutations	140
		Lama1 overexpression	Lama1 expression was induced in vitro and in vivo, can potentially be used for other attractive targets, eg agrin; assessment of functional effects lacking	141

Abbreviations: IGF-1, insulin-like growth factor 1; GTA, glatiramer acetate; FTS, farnesylthiosalicylic acid; C3, complement 3; 3-MA, 3-methyladenine; CT, cytotoxic T cell; PMO, phosphorodiamidate morpholino oligomer; EHS, Engelbreth-Holm-Swarm; ECM, extracellular matrix.

A potential issue associated with laminin- $\alpha 2$ therapy in LAMA2 MD patients is the induction of an immune response against laminin- $\alpha 2$ itself.⁵⁹ Most patients have no laminin- $\alpha 2$ since birth, and so any introduced laminin- $\alpha 2$ will be seen as foreign and may elicit an immune response. To overcome this, groups have looked into treating LAMA2 MD through laminin- $\alpha 1$ (*LAMA1*) overexpression. Out of all the α laminins, laminin- $\alpha 1$ is the most structurally similar to laminin- $\alpha 2$.^{72,73} However, laminin- $\alpha 1$ is not expressed in the adult neuromuscular system, being found mostly during early embryogenesis and having decreased expression in most adult tissues except the epithelia, kidneys, testes, and liver.^{21,73} Exogenous provision of laminin- $\alpha 1$ or activation of silenced *LAMA1* promoters in muscles and nerves is therefore necessary.

Gawlik et al (2004) produced transgenic dy^{3K}/dy^{3K} mice with the mouse *Lama1* cDNA driven by a cytomegalovirus (CMV) enhancer and a chicken β -actin promoter.⁷⁴ The dy^{3K}/dy^{3K} model was also created by targeted *Lama1* gene disruption, resulting in a complete deficiency of laminin- $\alpha 1$.⁷⁵ Severe dystrophic phenotypes and increased cell death were observed, accompanied by significant growth retardation and a shortened lifespan of typically less than 5 weeks. A transgenic dy^{3K}/dy^{3K} line that expressed *Lama1* the highest in skeletal muscle had significantly higher body weight, was as active as wild-type, and are fertile. Muscle basement membranes were restored, and dystrophic histopathology was ameliorated. Lifespan was increased to beyond 10 weeks, and a more longitudinal study⁷⁶ showed ~63% can survive up to 1.5 to 2 years while maintaining *Lama1* expression and restored skeletal/cardiac muscle morphology. A different transgenic dy^{3K}/dy^{3K} line that expressed *Lama1* in skeletal muscles and peripheral nerves was also studied and showed amelioration of neurological phenotypes (eg, demyelination).⁷⁷

Transgenic overexpression of *Lama1* likewise improved dystrophic phenotypes in dy^{2J}/dy^{2J} mice;⁷⁸ it may also positively affect fertility, as shown in dy^{3K}/dy^{3K} mice.⁷⁹ While promising, the laminin- $\alpha 1$ substitution approach is hindered by the large size of the *LAMA1* cDNA (~9.6 kbp). This size is difficult to package into viral vectors. *LAMA2* cDNA shares a similar size and faces the same issue. High-capacity adenoviral vectors overcome this size limit,⁸⁰ but their application remains to be tested for LAMA2 MD. Another option is electroporation, which has been done previously for *LAMA2*-containing plasmids,⁸¹ if its efficiency can be improved.

One way around this issue is by using protein therapy. Laminin- α 1, with laminin- β 1 and γ 1, forms laminin-111 in basement membranes and functions similarly as laminin-211.⁸² Laminin-111 provision may be a feasible treatment for LAMA2 MD. Intraperitoneal injections of laminin-111 (10 mg/kg/week) derived from Engelbreth-Holm-Swarm (EHS) mouse tumors were previously done in dy^W/dy^W mice.⁸² Treatment increased lifespan 3.5-fold, with a median survival at \sim 9.5 months compared to saline-injected controls at \sim 2.7 months. Forelimb strength, mouse activity, and muscle fiber count were significantly improved yet still significantly less than wild-type. Laminin-111 therapy can also improve the regenerative capacity of dy^W/dy^W cardiotoxin-injured muscles.⁸³ For this kind of therapy, however, an in-depth study of the pharmacokinetic characteristics of laminin-111 is recommended to ensure delivery and bioavailability. Excitingly for the field, laminin-111 has proven highly beneficial for the treatment of Duchenne muscular dystrophy (DMD), a related disorder caused by lack of the dystrophin protein and subsequent disruption of the DGC. With promising results in a large animal model,⁸⁴ it is likely that clinical trials testing laminin-111 for DMD treatment will soon be underway. This shows that therapies aimed at ECM restoration may not necessarily be limited to treating a single neuromuscular disorder, given the often-shared molecular pathophysiology of this group of diseases.

Use of linker proteins

Restoring interactions between laminins and cell surface receptors contributes substantially to the therapeutic efficacy, since these interactions mediate signaling between the ECM and adjacent cells, as well as help maintain membrane integrity. To treat LAMA2 MD, certain groups have instead focused on restoring or strengthening these interactions through linker proteins. The most studied linker protein for LAMA2 MD therapy is the miniaturized form of agrin or mini-agrin. Agrin is a heparan sulfate proteoglycan whose muscle-specific isoform has N- and C-terminal domains that bind laminins and α -dystroglycan, respectively.⁸⁵ Agrin is thought to be important for helping transmit forces between the basement membrane and the cortical cytoskeleton of muscle cells via the DGC.⁸⁵ Mini-agrin is composed of these N- and C-terminal domains, connected by one follistatin-like domain.⁸⁶ Laminin- α 4 is upregulated in LAMA2 MD and forms laminin-411 with the β 1 and γ 1 chains, but only weakly binds α -dystroglycan.^{86,87} It is expected that mini-agrin will help make laminin-411 a substitute for laminin-211.

Moll et al (2001) created transgenic dy^W/dy^W mice with a chick mini-agrin cDNA construct driven by the mouse MCK promoter.⁸⁶ Mini-agrin was correctly localized in basement membranes at high levels in skeletal muscles, but only minimally in the heart. Transgenic mice had generally improved health, with wild-type-like body weight and growth, as well as improved performance in the open field and rotarod tests. Survival was increased to at least 40 weeks. Myopathy was mostly non-evident in 4-week-old transgenic mice, but became more apparent in 16-week-old mice. CK activity was also significantly reduced in transgenic mice, yet still about thrice the levels observed in wild-type. Late-onset expression of the mini-agrin transgene had a similar effect.⁸⁸ Transgenic dy^{3K}/dy^{3K} Lama2-null mice with mini-agrin were made in a different study, and while improvements in muscle morphology and regeneration were observed, these were not as good as those displayed by transgenic dy^W/dy^W mice.⁸⁹

Mini-agrin treatment does not completely ameliorate LAMA2 MD pathology. This can be due to a number of reasons, one being insufficient delivery to target tissues. Many studies have investigated potential means of delivery including using adeno-associated viruses (AAVs) with serotypes 1, 2,⁹⁰ and 9,⁹¹ or using a combined cell- and gene-therapy approach with mesoangioblasts, which are mesodermal, blood vessel-associated progenitor cells.⁹² Thus far, use of AAV9 seems to be most promising in terms of treating both muscular and neurological phenotypes of LAMA2 MD.

Another reason for the reduced efficacy could be that laminin polymerization, which is not addressed by mini-agrin, is required for complete therapy. To remedy this, a fusion protein was made that consisted of the laminin α 1 LN polymerization domain at the N-terminus, and the nidogen-1 G2 and G3 domains at the C-terminus. The protein, called α LNNd, can direct laminin polymerization through the LN domain, bind laminin γ 1 via the G3 domain, and bind collagen IV via the G2 and G3 domains.⁹³ Studies showed that α LNNd can rescue the polymerization of mutant, N-terminal truncated laminins, such as those in dy^{2J}/dy^{2J} mice, which significantly ameliorated fibrosis and myofiber morphology, as well as improved forelimb grip strength.⁹⁴ Mice transgenic in both mini-agrin and α LNNd had more continuous basement membranes, less fibrosis, and more and bigger muscle fibers than single transgenic mice.⁹⁵ Muscle function, body weight, and survival were also better in double transgenic mice, yet there is room for improvement to

reach wild-type levels. Given such findings, exploration of other linker proteins, eg, those with both polymerization and cell surface receptor binding functions, would be worth looking into for therapy.

Adjusting integrin expression and activity

Approaches have also been developed to modify integrin expression for LAMA2 MD, as integrin dysregulation is a feature of the disease. Overexpression of the $\alpha 7$ integrin subunit was done by Doe et al (2011) in dy^W/dy^W mice by transgene introduction.⁹⁶ This led to enhanced localization of the $\alpha 7\beta 1$ integrin at skeletal muscle cell membranes and generally ameliorated muscle histology. Lifespan was prolonged 2.4-fold compared to non-transgenic dy^W/dy^W mice, accompanied by improvements in muscle function. Body weight, however, was not significantly increased by treatment.

A different group showed that a seemingly opposite strategy, ie, $\beta 1$ integrin inhibition, may be beneficial for LAMA2 MD treatment. Using a $lama2^{-/-}$ zebrafish model of LAMA2 MD, Wood et al (2018) reported that treatment with RGD peptide, a $\beta 1$ integrin receptor antagonist, significantly increased collagen deposition at the ECM and enhanced muscle fiber stability.⁹⁷ However, this did not lead to functional improvement as evaluated by a swimming test.

These studies highlight the complexity surrounding the role of the $\alpha 7\beta 1$ integrin in LAMA2 MD pathogenesis. More studies are needed to understand LAMA2 MD biology in this respect. Whichever approach is used, modifying integrin expression or activity does not appear to result in considerable alleviation of LAMA2 MD symptoms. The restoration of other laminin interactions at the basement membrane may be more important for treatment, or perhaps there are key regulators of integrin expression or alternative integrin isoforms, eg, αV and $\alpha 5$,⁹⁸ that have to be targeted for therapy. While challenging, combinational therapy of the various basement membrane treatment approaches is also an option and will likely result in increased efficacy.

Modulating downstream cellular events

Here, we primarily discuss treatments targeting cell growth and death, the immune response and fibrosis, as well as intracellular systems of regulation. Most of these approaches make use of pharmacological agents, which may have broad ranges of effect. The cellular events listed earlier also exhibit some degree of interdependence with

each other. Thus, while we attempt to categorize these treatments for ease of reading, their effects may not be limited to the group we place them in.

Targeting cell growth and death

LAMA2 MD is characterized by muscle wasting and, at the cellular level, reduced myofiber size and number. Treatment with insulin-like growth factor 1 (IGF-1) has been explored as a way to counter this issue. IGF-1 initiates pathways that promote cell growth, differentiation, and survival, eg, MAPK and PI3K signaling.⁹⁹ Lynch et al (2001) subcutaneously treated dy^{2J}/dy^{2J} mice with 2 mg/kg IGF-1 for 4 weeks and found that it significantly increased the cross-sectional area and mass of the extensor digitorum longus (EDL) and soleus (SOL) muscles.¹⁰⁰ Treated mice had significantly higher body mass than dy^{2J}/dy^{2J} controls; however, it did not reach wild-type levels. Kumar et al (2011) conducted a more in-depth study of the restorative effects of IGF-1 treatment by overexpressing it in dy^W/dy^W mice under control of the myosin light chain (MLC) promoter.¹⁰¹ Besides beneficial effects on growth and survival, the transgene also improved muscle regeneration, decreased apoptosis, and increased mouse activity. Additionally, systemic IGF-1 administration with human mesenchymal stromal cells has been shown to treat dy^{2J}/dy^{2J} mice well.¹⁰² Clenbuterol, a β -adrenergic receptor agonist and muscle anabolic agent, also ameliorated disease in the dy/dy model.¹⁰³

Occurring with reduced muscle growth in LAMA2 MD is increased muscle death. Studies show that interfering with the expression of genes involved in apoptosis can help treat LAMA2 MD. For instance, dy^W/dy^W mice that were either null for the pro-apoptotic *Bax* gene or overexpressing the anti-apoptotic *Bcl2* gene showed improvements in lifespan, growth, and muscle histology.^{104,105} Small molecules have also been used to inhibit apoptosis, including the (-)-deprenyl analog omigapil and the antibiotic doxycycline. Omigapil inhibits the GAPDH-Siah1-CBP/p300 apoptotic pathway, which is activated in dy^W/dy^W mice.¹⁰⁶ Omigapil treatment decreased the expression of apoptosis-related genes and the number of apoptotic nuclei in skeletal muscles. Treatment also led to improvements in overall health of the treated mice, and decreased the severity of skeletal defects. Omigapil also decreased fibrosis and improved respiration in dy^{2J}/dy^{2J} mice, but appreciable effects were not observed for muscle function.¹⁰⁷ Santhera Pharmaceuticals recently completed

a Phase I open-label clinical trial (NCT01805024) in 2018 testing the pharmacokinetic properties, safety, and tolerability of omigapil in CMD patients, including those with LAMA2 MD.¹⁰⁸ Results from the dose escalation study have not been published yet; however, the outcome seems favorable.

On the other hand, doxycycline ameliorated both muscle and nerve pathologies in dy^W/dy^W mice.^{109,110} Optimization of doxycycline therapy, given its adverse effect on angiogenesis and other cellular processes, remains to be achieved. Studies show that inhibiting apoptosis in tandem with other approaches, eg, IGF-1 supplementation¹¹¹ or mini-agrin introduction,¹¹² give enhanced benefit in LAMA2 MD mice. Combinational therapy can be an avenue to explore for LAMA2 MD, not only with treatments targeting apoptosis but for other treatments described in this review as well.

Targeting the immune response and fibrosis

The most-studied drug in this category is losartan, an anti-fibrotic agent. Losartan blocks the angiotensin II receptor, which inhibits activation of the transforming growth factor $\beta 1$ (TGF- $\beta 1$) pathway that promotes fibrosis.^{113,114} Angiotensin II also activates fibrotic pathways independent of TGF- $\beta 1$,¹¹⁴ which are inhibited by losartan. Losartan and its derivative L-158,809 have been shown to improve muscle strength, regeneration, and histopathology in LAMA2 MD mice.^{113,115} Interestingly, losartan also exerts effects on the MAPK and NF κ B pathways,^{113,116} as well as reverses dysregulation of the αV and $\alpha 5$ integrins in LAMA2 MD mice.⁹⁸ Combinational treatment of losartan with IGF-1 further enhanced its therapeutic efficacy.¹¹⁷ In 2016, the US Food and Drug Administration granted Orphan Drug status for TXA127 (Tarix Orphan) for LAMA2 MD treatment.¹¹⁸ TXA127 is a pharmaceutical equivalent of angiotensin (1–7), a naturally occurring peptide derived from angiotensin II cleavage that counteracts angiotensin II.^{118,119} Studies on TXA127 are yet to be published.

Chronic inflammation is typical in muscular dystrophies and poses numerous harmful effects besides fibrosis such as increased immune cell infiltration and hyperactivity, decreased muscle regeneration, and propagation of muscle death.^{120,121} Modulators of these processes have been investigated for their potential to treat LAMA2 MD, eg, prednisolone,¹²² glatiramer acetate,¹²³ halofuginone,¹²⁴ and farnesylthiosalicylic acid.¹²⁵ All have been found to ameliorate LAMA2 MD pathology to different extents. LAMA2 MD mice deficient in the expression of

complement 3, galectin-3, and osteopontin—genes that promote inflammation and/or fibrosis—have also been generated and examined. A spectrum of outcomes was observed: complement 3 deficiency proved beneficial for LAMA2 MD treatment,¹²² whereas loss of galectin-3 and osteopontin had negligible or surprisingly deleterious effects on disease progression, respectively.¹²⁶

Targeting intracellular systems of regulation

LAMA2 MD is also characterized by increased proteasomal activity, autophagy, and intracellular calcium levels. Inhibiting these processes has proven useful for treating LAMA2 MD. Systemic treatment of dy^{3K}/dy^{3K} mice with the proteasome inhibitor MG-132 significantly reduced fibrosis and apoptosis, as well as significantly increased overall muscle fiber size, mouse activity, and lifespan.¹²⁷ Systemic treatment with bortezomib, a more selective proteasome inhibitor, likewise resulted in significant improvements in the same model.¹²⁸ However, proteasome inhibition in general only achieves partial recovery at best and is not particularly useful for treating partial laminin- $\alpha 2$ deficiency.¹²⁹ The same can be said for the therapeutic efficacy of 3-methyladenine, an inhibitor of autophagy.¹³⁰ Early studies on handling high intracellular calcium levels in LAMA2 MD have been done and yielded positive results, eg, using caldecrin to reduce serum calcium concentrations¹³¹ and decreasing cyclophilin D expression to inhibit activation of calcium-mediated apoptosis.¹³² Besides these agents, metformin has also been found to reduce pathology and improve health in dy^{2J}/dy^{2J} mice, being generally more beneficial for females than males.¹³³ The mechanisms of action of metformin are uncertain, but it is suggested that it targets metabolism. Finally, overexpression of the cytotoxic T cell GalNAc transferase was also found to reduce dystrophic pathology in dy^W/dy^W mice, likely by influencing protein glycosylation or by promoting agrin expression in skeletal muscles.¹³⁴

Corrective genetic approaches to treat LAMA2 MD

Two corrective genetic strategies have been tested for LAMA2 MD: exon skipping and CRISPR/Cas9. A third with a somewhat similar purpose, premature stop codon readthrough using antibiotics, was attempted but did not restore laminin- $\alpha 2$ expression.¹³⁵

Exon skipping uses antisense oligonucleotides (AOs) to exclude selected exons from the final mRNA product

of a gene.¹³⁶ AOs bind pre-mRNA sequences via base-pairing at chosen sites to influence splicing. Target sites are usually splice sites or exonic splicing enhancers, the masking of which will cause the splicing machinery to skip certain exons. Exon skipping can restore the reading frame of mutant mRNAs created by out-of-frame deletions, as well as exclude out in-frame exons with nonsense mutations. Ultimately, this restores translation of truncated but partially functional proteins, as extensively shown for DMD.¹³⁷ To test this approach for LAMA2 MD, Aoki et al (2013) intramuscularly injected AOs of the phosphorodiamidate morpholino oligomer (PMO) chemistry into the TAs of dy^{3K}/dy^{3K} mice to skip *Lama2* exon 4.¹³⁸ Two PMOs were used (400 µg/kg), both targeting exon 4, which contained the disruptive neomycin cassette in the dy^{3K}/dy^{3K} model. Exon 4 skipping was induced, and up to 20% laminin-α2-positive fibers (presumably N-terminal truncated forms) were observed in the TAs of treated mice. Intraperitoneal injections of the same PMOs, at a total dose of 150 mg/kg, also resulted in exon 4 skipping and an improvement in lifespan compared to saline-treated controls. It is unclear if exon skipping therapy can lead to other forms of improvement, eg, muscle function, or if its efficacy for treating LAMA2 MD can be improved through enhancing oligonucleotide delivery.

On the other hand, CRISPR/Cas9 induces a more permanent form of correction, through targeted gene editing. The CRISPR/Cas9 system is essentially composed of the Cas9 endonuclease that can make double-stranded DNA breaks and a guide RNA (gRNA) that can direct Cas9 to where it can induce DNA cleavage.¹³⁹ CRISPR/Cas9 has been tested if it can correct the *Lama2* mutation in dy^{2J}/dy^{2J} mice. Kemaladewi et al (2017) designed gRNAs with Cas9 derived from *Staphylococcus aureus* (SaCas9) to delete the *Lama2* intron 2 region containing the splice site mutation through nonhomologous end-joining (NHEJ).¹⁴⁰ This leads to use of a different splice site downstream in the intron, which results in exon 2 inclusion and eventual successful translation of laminin-α2. Intramuscular, intraperitoneal, and temporal vein injections of these CRISPR components all led to *Lama2* exon 2 inclusion and restored laminin-α2 protein synthesis. Systemic treatment significantly reduced fibrosis and CNF count, and temporal vein injection in particular significantly ameliorated both muscular and neurological phenotypes to nearly wild-type status.

A variant of the CRISPR/Cas9 system can also be used to induce gene expression. Catalytically inactive Cas9 fused to a

transcription activation domain such as VP160 can be directed to promoters of selected genes and enhance gene transcription. Perrin et al (2017) showed that this increases laminin-α1 expression in vitro and in vivo.¹⁴¹ Whether this leads to functional improvement, however, remains to be determined.

Conclusion

Extensive progress has been made in understanding the multi-faceted, complex pathophysiology of LAMA2 MD since its initial characterization. Advances in research on laminin-α2 and its role in muscle have contributed largely to this development. We are also now beginning to more appreciate the functions of laminin-α2 in the heart, with cardiac involvement becoming increasingly represented as a feature of LAMA2 MD in the literature. ECM integrity is recognized as a vital contributor to heart structure and function, with pathological alterations to the ECM linked to cardiovascular disease.¹⁴² As laminin-211 is a major component of the myocardial ECM, it is likely that its loss will have important implications for cardiac physiology. Furthermore, while we only touched on it briefly here, LAMA2 MD also has a neurological component that primarily concerns the peripheral nerves. Studies developing therapies for LAMA2 MD are increasingly taking this into consideration in treatment or drug design, ensuring that both muscles and nerves benefit from the restorative effects of an approach.

On the subject of treatment, it is reassuring that numerous strategies have been and are being investigated for LAMA2 MD therapy. While some of these are moving closer to the clinic, eg, omigapil and TXA127, much work still needs to be done to improve therapeutic efficacy. It is often the case with these therapies that partial amelioration of LAMA2 MD pathology is achieved; rarely is the effect consistent, complete, and long-lived. More treatment optimization and research are recommended. As mentioned earlier, combinational therapy is an option. Other targets can also be selected for therapy. Various microRNAs are dysregulated in LAMA2 MD, and their expression can certainly be modulated as an approach.^{143,144} Polyamines,¹⁴⁵ decorin,¹⁴⁶ Ku70,¹⁴⁷ p53, and sirtuin¹⁴⁸ are also starting to be recognized as potential therapeutic targets. Managing diet¹⁴⁹ or bone marrow transplantation¹⁵⁰ may also be options, but follow-up studies into these are lacking. Therapies for other muscular dystrophies can also be adapted and tested for LAMA2 MD, as have been done for many of the strategies described. Overall, a combination of basic, translational, and clinical research efforts are

needed to ensure that we not only understand LAMA2 MD in its entirety but also know how to treat it, so as to provide patients with a cure as soon as possible.

Acknowledgments

This work was supported by Muscular Dystrophy Canada, The Friends of Garrett Cumming Research Fund, the HM Toupin Neurological Science Research Fund, Canadian Institutes of Health Research (CIHR) – 132574 and 143251, Alberta Innovates: Health Solutions (AIHS), Canada Foundation for Innovation (CFI), Alberta Advanced Education and Technology, and the Women and Children's Health Research Institute (WCHRI).

Disclosure

The authors report no conflicts of interest in this work.

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