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**European Joint Programme on Rare Diseases workshop:  
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therapy March 17–19, 2023, Barcelona, Spain**

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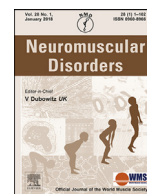
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## European Joint Programme on Rare Diseases workshop: LAMA2-muscular dystrophy: paving the road to therapy March 17–19, 2023, Barcelona, Spain

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### ABSTRACT

The European Joint Programme on Rare Diseases (EJPRD) funded the workshop "LAMA2-Muscular Dystrophy: Paving the road to therapy", bringing together 40 health-care professionals, researchers, patient-advocacy groups, Early-Career Scientists and other stakeholders from 14 countries. Progress in natural history, pathophysiology, trial readiness, and treatment strategies was discussed together with efforts to increase patient-awareness and strengthen collaborations. Key outcomes were (a) ongoing natural history studies in 7 countries already covered more than 350 patients. The next steps are to include additional countries, harmonise data collection and define a minimal dataset; (b) therapy development was largely complementary. Approaches included LAMA2-replacement and correction, LAMA1-reactivation, mRNA modulation, linker-protein expression, targeting downstream processes and identifying modifiers, using viral vectors, muscle stem cells, iPSC and mouse models and patient lines; (c) LAMA2-Europe will inform patients (-representatives) worldwide on standards of care and scientific progress, and enable sharing experiences. Follow-up monthly online meetings and research repositories have been established to create sustainable collaborations.

### 1. LAMA2-MD: Paving the road to therapy

From March 17 to March 19, 2023, more than 40 experts (clinicians, scientists and patients and their representatives) from 14 countries gathered onsite in Barcelona, Spain, and many more online (108 participants from 28 countries). The experts presented and discussed progress in the knowledge on natural history, pathophysiology, trial readiness and potential treatment strategies for patients with LAMA2-related muscular dystrophy (LAMA2-MD), the congenital form being known as merosin deficient congenital muscular dystrophy type 1A (MDC1A; OMIM #607855). LAMA2-MD is an ultra-rare, clinically heterogeneous neuromuscular disease, affecting 1 in 30,000–100,000 individuals. It is caused by pathogenic mutations in the LAMA2 gene, which encodes laminin- $\alpha$ 2, a component of heterotrimeric laminin-211,

also known as merosin. Laminin-211 is expressed in basement membranes of many tissues, for example – striated muscle, placental trophoblasts, Schwann cells, neuromuscular synapses and brain cells, but also in the thyroid gland, adrenal gland, testis and stomach. Complete loss of laminin-211 results in disruption of structural stability and signal transduction of the extracellular matrix (ECM). The spectrum of LAMA2-MD ranges from a severe phenotype presenting with hypotonia, muscle weakness, contractures at birth or the first months of life (complete deficiency), to a milder (partial deficiency) late-onset 'Limb Girdle Muscular Dystrophy'(LGMD)-like phenotype. About 90 % of the cases belong to the congenital-onset severe phenotype. Affected children reach motor milestones later and more than 90 % do not achieve the ability to walk independently. Children may also develop muscle contractures which restrict the movement of their limbs and joints. Many children with LAMA2-MD also develop chest deformities and scoliosis. Weakness of the respiratory muscles can result in frequent, sometimes fatal chest infections and poor breathing at night. Severe cardiac involvement is not expected in LAMA2-MD, although rhythm abnormalities have been

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described. Around 30 % experience seizures, typically responding well to antiepileptic medications. Partial loss of laminin-211 leads to much milder disease manifestations and independent walking is typically achieved. However, the clinical course and deterioration of muscle weakness of LAMA2-MD is not fully known, and, despite intensive research, a cure is not available. Also, existing treatments to preserve quality of life do not reach all patients and clinicians equally [1].

The objective of the conference was to address these issues and make further steps on the road to therapy for LAMA2-MD. Specific aims were 1. Aligning patient registries and natural history studies and defining biomarkers and guidelines which can be used in clinical care; 2. Defining diagnostic criteria and guidelines for alleviating symptoms and maintaining quality of life with a dissemination plan for Europe (and globally); 3. Listing available samples, biomaterials and protocols, sharing confidential progress on therapy research and establishing a LAMA2-MD therapy taskforce. The workshop was unique thanks to a continuous and equal involvement of clinicians, researchers, patients, and patient organisations (onsite and online) before, throughout and after the event. The workshop was also timely and needed, as awareness of LAMA2-MD is increasing, natural history studies have started and therapeutic possibilities are emerging, whereas many patients are still outside reach, lacking a diagnosis or prospective and adequate symptomatic treatment.

The workshop report starts with the patient perspective (Section 2), which was organised by the patient organisations for patients and their caregivers. The patient organisations had chosen the topics and the speakers, which presented in English at a level which everybody would be able to understand. This was followed by break-out sessions in multiple languages, in which national representatives were doing the translations. The report continues with a summary of the scientific lectures (Sections 3–5) and ends with a wrap-up and action plan of the clinicians, researchers and patient organisations to make the outcomes and efforts sustainable (Section 6).

## 2. The patient perspective: a general overview

Reghan Foley (NIH Bethesda Maryland, USA) presented a general overview of the clinical manifestations of LAMA2-MD and characteristic findings and course of the disease, followed by a discussion on clinical trial readiness covering three issues. The first issue involved the optimization of clinical care, for which Foley specifically addressed respiratory, cardiac, and orthopaedic surveillance, as well as feeding and nutritional care. The second issue concerned the identification and application of clinical outcome measures, like Motor Function Measurement (MFM) or muscle imaging, and of biomarkers from serum, urine and/or muscle tissue that can reflect potential therapeutic changes [2,3]. Finally, Foley emphasised the importance of natural history studies, in which non-invasive ventilation for pulmonary function enhancement should have a central place.

Andrea Klein (University Hospital Bern, Switzerland) presented an overview of ongoing natural history studies for LAMA2-MD in different countries. She emphasised the necessity of conducting these studies in LAMA2-MD, both nationally and internationally, to acquire more information about prognosis and possible complications, to develop measures for avoiding and treating complications early, and to achieve a faster diagnosis in patients that do not have a «typical phenotype». This will lead to more detailed and more harmonised standards of care. To collect the necessary information, she stressed the importance of developing an international consensus on a core dataset for registries and natural history studies. She concluded her

presentation by describing the challenges and proposed ways for collaboration and support.

Céline Damon and Séverine Berreur (LAMA2 France) presented the founding of LAMA2 Europe, a European consortium of patient organisations, initiated by VoorSara, ImpulsaT and LAMA2 France. They discussed the objectives of this European collaboration, which include facilitating potential trials within Europe, supporting and co-funding international research, and, primarily, informing patients and families of the latest research progress in the field. Their activities included increasing the visibility of LAMA2-MD and collaborating closely with scientists exemplified by co-organizing the workshop in Barcelona. They encouraged patients, relatives, and patient organisations to join LAMA2 Europe and expressed their commitment to support the LAMA2-MD researchers and clinicians alike.

Susana Quijano-Roy (University of Versailles-St Quentin/Garches Raymond Poincaré Hospital, France) presented pulmonary care in LAMA2-MD patients and respiratory involvement in neuromuscular disease, drawing upon research conducted at Garches Hospital, involving 40 patients. The significance of frequent assessment of respiratory function was emphasised, and several tests were discussed, including spirometry, blood gas analysis, or night studies like polysomnography or oxycapnography. Moreover, the assessment of spinal function was stressed as a crucial aspect of the identification of complications at early stages. Additionally, Quijano-Roy highlighted the importance of implementing daily care measures focusing on both respiratory functions and management of scoliosis by means of: (1) Respiratory therapies such as hyper-insufflation and abdominal belt, daily sessions or proactive nocturnal non-invasive ventilation (to enhance thoracopulmonary growth); (2) Axial stretching physical therapy and spinal orthosis (braces) respectful with respiratory function (Garches brace) to limit progression of scoliosis; (3) Orthopaedic spinal interventions to reduce stiff severe spinal deformities before the confection of braces or spinal surgeries (casts, cephalic halo-traction); (4) Spinal surgery techniques (spinal fusion vs new growing rod implant techniques), aimed at limiting respiratory progression and improving prognosis in the long term. These proactive and preventative respiratory and orthopaedic approaches may change natural history of the disease and, therefore, the studies and registries should include detailed information about clinical management in order to be able to capture real data.

Clara Gontijo Camelo (University of São Paulo, Brazil) presented therapeutic interventions for maintaining quality of life of LAMA2-MD patients. She addressed the functionality of orthoses, parapodia and extension boards in preventing scoliosis and contractures. Moreover, she stressed the importance of bone health via calcium, vitamin D and alendronate supplementation to avoid fractures and minimise risks of surgical complications in a scoliosis correction. Additionally, the importance of speech therapy for LAMA2 patients with tongue hypertrophy and feeding difficulties was addressed. Lastly, Gontijo Camelo highlighted orthodontic and nutritional care in relation to hypoglycemia [4]. Finally, she addressed the sleeping problems commonly experienced by the patients, due to limited nocturnal mobility.

Madeleine Durbeej (Lund University, Sweden) first explained the underlying mechanisms responsible for the manifestation of LAMA2-MD symptoms. Next, she discussed the therapeutic strategies currently being investigated. Targeting the primary genetic defect has shown a clinical improvement in LAMA2-MD mouse models. Modulating cellular events caused by laminin  $\alpha 2$  chain loss was introduced as a supporting treatment. Furthermore, Durbeej clarified the current advancements in research on gene correction with CRISPR-Cas9-mediated genome editing and

highlighted the progress made in the development of cell therapy for LAMA2-MD.

### 3. Clinical manifestations, clinical history and trial readiness

Andrea Klein (University Hospital Bern, Switzerland) presented results from a cross-sectional cohort and ongoing national history study for LAMA2-MD in Switzerland. Patients were recruited via the Swiss registry for neuromuscular disorders. The Swiss LAMA2-MD cohort consists of 17 patients, but the population might be larger, as mild cases and adult patients might not all have been detected or diagnosed. The age at onset of clinical symptoms was generally within the first year of life. Brain Magnetic Resonance Imaging (MRI) showed structural changes in a large proportion of patients, but these were not related to cognitive abilities or epilepsy. Only one patient had no white matter changes, but MRI was performed in the neonatal period. The majority of patients were able to hold their head up and achieved the ability to sit. Motor function tests revealed that three patients were able to walk and perform almost every task. Preliminary results indicated that assessment by Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP Intend) and Motor Function Measurement (MFM) did not change much within the first year of the study and changes in motor scores were influenced by development in young children and interventions, like scoliosis surgery in older children. The need for larger cohorts, consensus on outcome measures and biomarkers, patient reported outcome measures and standardised methods for measuring contractures including rigidity of the spine and neck flexion, was highlighted.

Francina Munell (Hospital Universitari Vall d'Hebron) reported on the ongoing prospective natural history study in Spain. A total of 20 patients had been included so far. Except for one patient, age of onset was within the first three months of life and symptoms at onset included hypotonia. Age of sitting acquisition and head control correlated. All patients had normal cognition. Genetic analysis revealed that 14 patients carried frameshift variants, 8 patients nonsense variants, 5 patients splicing variants and one patient two large deletions. The same pathogenic mutations were present in multiple patients within the Spanish cohort, but also in patients from other countries. The aims of the project include analysing clinical and imaging data, studying genetic variants and the epidemiology of LAMA2-MD, implementing electronic data collection (REDCap), promoting participation in international registries, and fostering collaborations focused on clinical studies and advanced therapies.

Reghan Foley (NIH Bethesda Maryland, USA) presented the results of an international retrospective study analysing natural history until the age of 5 years. Data was derived from medical records submitted to the Congenital Muscle Disease International Registry. The total cohort consisted of 60 patients. Congenital/early hypotonia, poor head control, muscle weakness, feeding difficulties, arthrogryposis, hip dysplasia, and joint contractures were initial symptoms at onset of the disease. There was a significant trend of declining creatine kinase levels in the first year of life. A total of 24 patients needed non-invasive ventilation by the age of five years. Reghan Foley also presented the planned LAMA2-related dystrophy Early Outcome Measures and Natural History Study (LEON), aiming to recruit 44 patients in the US for a prospective study lasting 5 years. A third study, the CMD Common Outcome Measures Study involved patients aged 4 to 22 years and spanned 4 years. Total MFM32 scores declined by 2.6 per year. Minimal Clinically Important Difference in the MFM32 was 2.5 percentage points applying within patient analysis and 3.9 percentage points between patient analysis. Clinical outcome assessments were completed in clinical and remote environments in regular care,

like the Neuromuscular Gross Motor Outcome (GRO) in children under the age of five years. Remote monitoring enables gathering of motor data in the natural environment and when validated can be used to monitor progress of congenital muscle diseases. Biomarkers are fundamental, but potential biomarkers as muscle thickness and echogenicity require further quantification and validation to serve as a biomarker. Quantitative MRI might serve as a potential biomarker of disease progression.

Karlijn Bouman (Radboud UMC, Netherlands) presented the first results of the ongoing LAST STRONG natural history study in the Netherlands. Twenty-seven patients were recruited in the age range of 3–50 years. Total MFM-20/32 scores were  $42.1 \pm 29.4$  % and correlated negatively with sedentary activity. Overall, patients with absent merosin expression had lower scores than patients with partial merosin expression. LAMA2-MD patients experienced reduced physical functioning and general health, fatigue, reduced quality of life, frequent pain and reduced social participation and work. Impaired pulmonary function was present in 85 % of patients, and 37 % needed respiratory support. Diaphragm thickness was normal in all but one patient [5]. No change in respiratory function was observed between baseline and 1.5-year follow-up. Cardiac function was impaired in some patients. Many showed cardiac risk factors, implying that cardiac follow-up should be performed. A total of 86 % of patients had low mineral bone density, though no change was observed between baseline and 1.5-year follow-up. Still, for optimising bone quality, vitamin D and calcium should be supplemented [6].

Göknur Haliloğlu (Hacettepe University, Turkey) gave an overview of LAMA2-MD patients in Turkey. A total of 40 LAMA2-MD patients with a mean age of 13.9 years were followed from 2011 to 2020; 38 patients had complete deficiency and two patients had partial deficiency. A high prevalence of consanguinity was observed (80 %). Age at onset was at birth for half of the patients, for the remaining patients it was within the first 5 months of life. A total of 70 % of patients had hypotonia and weakness, nearly all others displayed developmental motor delay. Cognitive development was normal in 20/40 of patients evaluated. Nine of 16 patients had a normal echocardiogram. EMG was neuropathic in 3/6 of patients assessed. Cranial MRI showed nonspecific changes in 3 out of 17 patients. There were 6 patients assessed between 2020 and 2022, all with complete merosin deficiency. Five of the 6 patients were younger than two years of age, and 2 out of these 6 patients were referred primarily due to demyelinating neuropathy highlighting the neuropathy component of the congenital-onset form. Challenges in Turkey included the lack of a national registry and lack of follow-up due to fragmented health-care systems and infrastructure. Although diagnosis of the historical cohort was mostly based on clinical, muscle histopathology and immunohistochemical studies, the diagnosis was molecularly confirmed. Göknur Haliloğlu also mentioned the potential of Hacettepe University in terms of targeted and untargeted metabolomics for biomarker studies. The aim is to have a Congenital Muscular Dystrophy Turkish Registry together with the Ministry of Health and CMDTR-Patient Advocacy Group, which can be implemented to international registries. This will not only help to increase awareness in terms of recent progress in the field but also improve diagnostic facilities and adherence to standards of care.

Susana Quijano-Roy (University of Versailles-St Quentin/Garches Raymond Poincaré Hospital, France) reported on LAMA2-MD natural history studies in France. In 2004, 29 patients with a mean age of  $15 \pm 8$  years were assessed. Progressive restrictive respiratory insufficiency was present in most children, with 62 % requiring mechanical ventilation. Most complications were related to retractility, joint and spinal deformities and thoracic cage stiffness or restriction. The cohort could be divided into two



distinct prognostic groups. The first group consisted of patients who were only able to sit independently and who exhibited more severe symptoms, including restrictive respiratory insufficiency before adulthood. The second group consisted of patients with a milder phenotype, and a better respiratory prognosis if spinal deformities were controlled. Nocturnal hypoventilation was observed in ventilated patients but also sometimes in patients with relatively good respiratory function suggesting possible additional aetiologies other than respiratory muscle weakness (CNS dysfunction, night brainstem dysfunction, ORL obstruction, airway obstruction). A study in 2022 with 38 patients showed that bronchial compression due to thoracic hyper-lordosis (vertebral penetration in the thoracic cage) occurred in at least 15 % of the recent cases, more frequently in non-ambulant patients with pronounced axial hypotonia. Daily hyper-insufflation therapy (20 min a day) was possible since the second year of life, and using an abdominal belt to reduce the aerophagia and optimise the thoracopulmonary expansion markedly improved the progression of the pulmonary function tests (FVC) and mouldet (rounded) the flat thorax appearance. In addition, management of scoliosis helped to reverse respiratory insufficiency, if the brace was respectful with respiratory function (Garches brace, Cheneau brace), or if the surgical treatment was adapted (fusion-less growing rods with no fixation in ribs). Less severe forms and atypical cases have been increasingly identified. Improvement in prognosis was observed, due to impact of clinical practices and this should be captured in natural history studies.

Andrea Seferian (Institut de Myologie, France) reported on a prospective, longitudinal, interventional natural history study that is going to be launched in 2023–24 in France, sponsored by AFM. A total of 40 patients between 2 and 15 years are expected to participate. Patients will be assessed for 24 months. The study will assess motor function, respiratory function and use of respiratory support, patient and parent reported outcome measures and cognitive assessment depending on the patient's age. Additionally, qualitative, and quantitative biomarkers using nuclear magnetic resonance will be investigated.

Anna Sarkozy (Dubowitz Neuromuscular Centre, UK) presented results of a retrospective natural history study for LAMA2-MD patients, 42 with complete merosin deficiency and 4 with partial deficiency, seen at the Dubowitz Neuromuscular Centre until 2019 [7]. Peri/neonatal symptoms were more common in patients with complete deficiency. Forceps delivery, contractures at birth, facial weakness and torticollis were observed only in such patients. All patients had motor delay. Patients with complete deficiency showed increased frequency of chest infections. Forced Vital Capacity (FVC) was  $<60\%$  in  $92\%$  and  $<40\%$  in  $56\%$  of patients at time of first evaluation. An annual decline in FVC% predicted of  $2.9\%$  was observed. Elbow flexion contractures  $>30^\circ$  was noted in  $76\%$  of patients with complete deficiency and in none with partial deficiency. Elbow flexion and knee flexion contractures showed a linear increase of  $6.6^\circ/\text{year}$  and  $3.1^\circ/\text{year}$ , respectively. Scoliosis was present in 33 patients with complete deficiency and in one patient with partial deficiency. The mean age of onset of scoliosis was 6.8 years, with  $80\%$  of patients developing scoliosis by the age of 10 years. Epilepsy was observed in  $10\%$  of patients with complete deficiency.

Stefano Previtali (San Raffaele Scientific Institute, Italy) discussed the initiative to get funding for a transnational natural history study and liquid biomarker identification in patients with LAMA2-related disease (TRACE LAMA2). The study is scheduled for three years with an evaluation every 6 months, aiming to recruit 100 patients, of which 60 have a severe phenotype and 40 a milder phenotype. The aim is to identify biomarkers and to determine the 24/30-months disease trajectory of selected outcome measures. Secondary aims are to determine the prevalence and severity of

the involvement of other organ systems, to correlate biomarkers with disease progression, severity, and phenotype and to calculate the sample size required to detect changes of parameters identified in this study. The data collected will be compared with data from LAMA2 deficiency mouse models. Advantages of this project would be the large cohort of patients from different countries, harmonised data in the participating countries, comparison with animal models and the strengthening of the interaction between clinicians, scientists, and patient advocacy organisations.

#### 4. Genotype-phenotype correlations, biomarkers, and models

Andreas Roos (University of Bochum, Germany) presented tissue related biomarkers for laminin-211 deficiency. Proteomic profiling via LC-MS/MS had been done on skeletal muscle in a cohort of 6 patients with pathogenic LAMA2 mutations. The results indicated a significant upregulation of 35 genes, including LAMA4, AGRN and GPC1, as well as the downregulation of 51 genes, including the gene NNMT, nicotinamide N-methyltransferase. Validation studies on the distribution of the downregulated genes highlighted the increased vulnerability of the mitochondrial respiratory chain in LAMA2-MD patients. NNMT is believed to play a major role in this dysfunction, as it inhibits NAD<sup>+</sup> biosynthesis. Proteomic profiling on laminin-211 deficient chorionic villi also showed the upregulation of NNMT, as well as CD97, a biomarker for inflammation in the early stages of the disease. The myopathological relevance of periostin, a fibrosis biomarker, was also examined, and its depletion was shown to ameliorate the dystrophic phenotype in mice. Immunostaining studies on periostin and CD90 showed that fibrosis often occurred not only by the activation of fibroblasts, but also in the muscle fibres.

Jacek Stepniewski (Jagiellonian University, Poland) presented the disease models he created, using human-induced pluripotent stem cells (hiPSCs). Peripheral blood mononuclear cells were reprogrammed using non-integrating Sendai vectors, and were successfully differentiated into skeletal muscle cells, cardiomyocytes, motor neurons and endothelial cells. CRISPR-Cas gene editing was used to create a cellular LAMA2 knockout model. Global transcriptomics analysis determined the upregulation of 691 genes, and the downregulation of 363 genes. Involved pathways included the PI3K-Akt pathway, cell adhesion molecule function, and ECM receptor interactions. The lack of laminin-211 led to a significant downregulation of PAX7, which controls the satellite cell phenotype, and an increased expression of HO-1, which regulates satellite cell differentiation.

Veronica Pini (UCLUniversity College London, UK) presented work on characterising modifier genes that could ameliorate LAMA2-MD severity. She presented 2 sibs with the homozygous frameshift mutation 8075-8076delGT in exon 56 of the LAMA2 gene, which resulted in a premature stop codon at position 2706. Despite having the same mutations, the siblings had different phenotypes, one being mild and the other one severe. Veronica Pini searched for modifiers by muscle RNASeq in both patients. A total of 710 genes were differentially expressed. The top 10 upregulated pathways mainly involved metabolic processes and the top 10 downregulated pathways inflammation and fibrosis. She is currently functionally testing the 3 major candidate genes in zebrafish and mouse models. In addition to this, whole genome sequencing will be performed as an alternative strategy to identify DNA variants that could explain the difference in clinical manifestation.

Clara Gontijo Camelo (University of São Paulo, Brazil) presented results from a retrospective and prospective non-interventional longitudinal study of 63 patients in Brazil from 2018 to 2022. Brain MRI from 52 patients showed that severe muscle involvement correlated with severe white matter abnormalities.

10 patients had brain malformations in the occipital lobe, but also in the frontal lobe and temporal lobes. 10 patients suffered from epilepsy, of which 9 had brain malformations. Of the 9 patients with an intellectual disability, 5 had brain malformation [8]. Gontijo Camelo reported a new homozygous frameshift mutation (C.1255del//p.Ile419\*4) in 11 patients without brain malformation, epilepsy, or intellectual disability. Another 7 patients were homozygous for the c.246A>C mutation. All of them were able to walk and had no brain malformation, epilepsy, or intellectual disability. A patient with a homozygous c.4960-17C>A mutation presented the most severe phenotype with brain malformation and intellectual disability. None of the patients with brain malformation were able to walk. Variants leading to brain malformations usually affected the laminin G (LG) domain. A total of 15 out of 48 patients had at least one episode of hypoglycaemia, 8 patients had more than one episode. Patients with hypoglycaemia had higher chances of loss of function mutations and needed non-invasive ventilation or gastrostomy more often.

Ana Rita Goncalves (Centro de genética médica doutor Jacinto de Magalhães, Porto, Portugal) presented the diagnostic strategies and mutational/phenotypic profile of a comprehensive cohort of Portuguese LAMA2-MD patients. The cohort consisted of 86 patients from 80 families. Seven pathogenic variants were found in the majority of the cohort (74 patients from 68 families), with frequencies ranging from 7 % to 25 %. A deletion of exon 56 was found in 15 families, and a patient homozygous for this deletion presented a more severe phenotype LAMA2-MD. The 2461A>C (p.Thr821Pro) pathogenic variant was found in 15 families. Based on the genetic data available, bioinformatics analysis and functional follow-up more precise genotype-phenotype correlations should be established, enabling a deeper understanding of LAMA2-MD aetiology. Finally, a deep-intronic variant c.2096+118A>G was found in intron 14, creating a novel splice-donor site, leading to an in-frame intron retention. As also wild-type mRNA was still visible, this mutation might be amenable for splice modulation.

Tim Nebermann (UM, The Netherlands) presented data on a Dutch founder mutation with aberrant splicing and variable clinical phenotype. The splice site mutation c.5562+5G>C was present in about half of the Dutch patients, either homozygous or compound heterozygous. Based on marker analysis it was demonstrated that the mutation originated from the same founder. As clinical manifestations varied from mild to severe in the homozygotes, Tim Nebermann explored the pathogenic mechanism. Three RNA isoforms were observed in fibroblasts and mesoangioblasts, two of which retained part of the intron, leading to a frameshift and premature stop codon. One transcript showed skipping of exon 38, which did not disturb the reading frame and led to a protein with an internal deletion. As protein studies at UCL had demonstrated the presence of merosin in such a homozygous patient, the shorter protein could be functional and that the level might explain the clinical variation. A splice-modulation strategy is now being explored to increase the amount of this protein.

## 5. New roads to therapy

Carsten G. Bönnemann (NIH, USA) gave an overview of the gene-therapy toolbox that could be applied to LAMA2-MD. One of the tools included (AAV mediated) gene replacement, for which the size of the LAMA2 cDNA was still a hurdle, but nanoparticles were an emerging alternative. If the full gene cannot be replaced, other smaller proteins might do the job. Linker proteins have been developed, which by connecting to LAMA4 can replace the defective LAMA2. CRISPR/Cas9-based reactivation of LAMA1 was another successful strategy in LAMA2 mouse

models. For correcting the mutations several strategies could be applied, being CRISPR/Cas9, prime editing or base editing, but though promising the efficiencies were still limited. Finally, for a subgroup of mutations splice modulation might be suitable. Although considerable progress was made there still are challenges facing us. These are related to the large size the cDNA (9369bp coding sequence), the broad variety of mutations, the tissues/cell types to target and correct, the timing of successful intervention, the level of correction/intervention needed, the durability of correction/intervention and the readouts.

Valérie Allamand (Sorbonne University, Inserm, France) and Leslie Caron (Aix-Marseille University, France) focused on generating new *in vitro* cellular models for LAMA2-MD to test new therapeutic strategies and identify biomarkers. iPSC lines from patients with missense and nonsense LAMA2 mutations will be differentiated into 2 populations: pure skeletal muscle fibres and innervated muscle fibres [9,10]. Transcriptomic analysis showed a downregulation of neuronal differentiation, a compromised structure and organisation of the sarcomeres, as well as reduced contractility in LAMA2 deficient muscle fibres. An AAV delivered Anticodon engineered tRNAs will be developed for the correction of nonsense LAMA2 mutations. The results from a prior study showed a significant advantage of this technique over gentamicin in terms of translational readthrough [11]. Because of the need for non-invasive ways to follow disease progression and treatment response, they propose to explore plasma profiling by nanoDSF [12], which uses machine learning to characterise and monitor new biomarkers.

Jordi Barquiner (University Hospital Vall d'Hebron, Spain) presented his approach to express the laminin-211 protein in the liver using a gutless adenoviral vector encoding human LAMA2 (made by the group of Ruben Hernandez, U. of Navarra), which was expected to be transported to muscular ECM via circulation. In hepatocytes, the results indicated successful delivery of LAMA2 at the DNA and RNA levels, but the protein was not detected. The reason could be that the liver cells did not consistently express the  $\beta 1$  and  $\gamma 1$  subunits needed to produce the heterotrimer. They checked for other potential target tissues which expressed the different subunits. Mesenchymal stem cells (MSCs) might be suitable, as they can be easily obtained from many different tissues and can be generally modified using *ex vivo* procedures. Mesenchymal cells from eGFP mice will be expanded *in vitro* and exposed to different conditions to see which ones are optimal for laminin-211 secretion. Next, the cells will be injected into *dy*<sup>2j</sup> mice and different parameters measured, such as biodistribution, long-term persistence and laminin-211 expression.

Madeleine Durbeek (Lund University, Sweden) presented skeletal muscle metabolism as a pharmacological target in LAMA2-MD. Mitochondrial respiration was significantly reduced in human LAMA2-MD myotubes and restored by adding laminin-211 [13]. LAMA2-MD muscle had increased ROS production. These findings suggested that skeletal muscle metabolism could be a pharmacological target to ameliorate disease manifestations. Metformin and N-acetylcysteine (NAC) enhanced grip strength in *dy*<sup>2j</sup>/*dy*<sup>2j</sup> mice. Metformin improved muscle histology in female mice. NAC reduced central nucleation and decreased the occurrence of apoptosis, inflammation, fibrosis, and oxidative stress in *dy*<sup>2j</sup>/*dy*<sup>2j</sup> muscle. Furthermore, vitamin E reduced central nucleation, inflammation, and ROS levels in muscle [14]. Madeleine Durbeek also presented work on the roles of other laminin chains in smooth, cardiac and skeletal muscle. Mice devoid of laminin  $\gamma 1$  in the cardiovascular system displayed lung and cardiac defects and a global and inducible mouse model of laminin  $\gamma 1$ -deficiency presented intestinal dysfunction and reduced smooth muscle contractility. Double knockout of laminin  $\alpha 2$  and  $\alpha 4$  induced severe muscular dystrophy in mice.

Dwi Kemaladewi (University of Pittsburgh, USA) presented CRISPR-Cas-based upregulation of the LAMA1 gene, which would compensate for the dysfunctional or absent LAMA2 protein. In  $dy^{2j}$  mice, AAV delivery of transcriptional regulators, targeting the LAMA1 proximal promoter domain, showed amelioration of dystrophic phenotypes, both in muscular and neuronal tissue. This approach was redesigned, so that the components were miniaturised and could fit in 1 virus, reducing toxicity. A  $dy^w$  mouse was treated this way and showed a significant improvement in survival, grip strength and muscle contractility, as well as an increase in body weight compared to the wildtype. LAMA1 upregulation was also achieved in human fibroblasts of both healthy subjects and LAMA2-MD patients.

Florence van Tienen (Maastricht University, The Netherlands) presented a myogenic stem cell therapy, using autologous mesoangioblasts (MABs) that can either express wild-type LAMA2 or LAMA1. The approach is to isolate MABs from the muscle of a subject with LAMA2 deficiency, perform genetic correction of LAMA2 or upregulation of LAMA1 in MABs *in vitro*, and expand and deliver them to the same subject via the arteries. The therapeutic agent, healthy autologous mesoangioblasts, was tested in a mitochondrial myopathy patient cohort (m.3243A>G mutation) for safety and partial efficacy. In this phase I/II clinical study (EudraCT 2016-001258-16), safety of autologous MAB administration was demonstrated, as no serious adverse events, vascular obstructions or changes and neurological vital signs were observed. One adverse event was observed, and all participants experienced some discomfort during the procedure. Four weeks after administration, signs of muscle regeneration were apparent in MABs-treated muscle.

Markus Rüegg (University of Basel) reported on the use of linker proteins in LAMA2 deficiency to re-connect the muscle cytoskeleton and the basement membrane. His group constructed 2 proteins that could serve this purpose: mini-agrin (mag) and  $\alpha$ LNNd. They bind to the Laminin 411 protein, which is upregulated in LAMA2-MD. The linker proteins can be delivered by the AAV system. If these linker proteins are expressed in the LAMA2 KO ( $dy^w/dy^w$ ) mouse model, a vast improvement was observed in the ECM, muscle histology and lifespan, with some mice living as long as wild-type mice. This approach, called Simultaneous Expression of Artificial Linkers (SEAL), was tested by injecting two AAVs that express either mini-agrin or  $\alpha$ LNNd in the muscles of LAMA2 KO ( $dy^w/dy^w$ ) mice. The body and muscle weight were significantly increased in the LAMA2 KO mice treated with the linkers, muscle histology strongly improved, and inflammation was significantly decreased. If the linker proteins were additionally expressed in the peripheral nerve also the peripheral neuropathy was prevented in LAMA2 KO mice.

Peter Yurchenco (Rutgers State University, USA) is part of the collaboration on the linker protein research. Transgenic expression of both linker proteins separately improved muscle physiology in both  $dy^{2j}$  mice and  $dy^w$  mice. Some domains of the  $\alpha$ LNNd linker were removed to make room for a larger and stronger promoter. The combination of the new protein  $\alpha$ LNNd $\Delta$ G2' and nidogen led to more laminin accumulation in myotubes and improved collagen IV recruitment.

Marc Güell (Pompeu Fabra University, Spain) tackled the limited insert size, when using AAVs and other gene delivery vectors. As the LAMA2 gene and protein are large, transgenesis would only be possible with a new delivery system. Find and cut-and-transfer (FiCAT) combined Cas-9 localization of the desired module, and an engineered transposase to deliver the insert from a plasmid, increasing the packaging capacity of the vector system from 4.7Kb to 11Kb. Güell's team managed to efficiently program transposases, making them suitable for mammalian systems. Mouse models, in which the LAMA2 cDNA was transferred showed a significant

increase in survival and strength. FiCAT can also be used for more efficient linker protein delivery and is a potential improvement for CRISPR-Cas-based LAMA2 gene correction, and upregulation of Laminin-111.

## 6. The next steps

The conference ended with a wrap-up and action plan by the patient organisations, clinicians and researchers to define targets and organize collaborative follow-up online meetings to make the outcomes and efforts of the workshop sustainable and speed up the road to therapy for LAMA2-MD.

- LAMA2-MD patient organizations and networks

After its successful start with several key countries, the patient organisation LAMA2-Europe aims to connect to all patients across Europe and have parents as ambassadors in each country. As follow-up, the network will be extended globally to include patients from all continents. The network will be used for communication to inform patients and their parents on the (regularly updated) standards of care for LAMA2-MD and on scientific progress. There will also be ample attention for emotional and psychological guidance of the patients and their families. Vice-versa, LAMA2-Europe wants to help the researchers in their efforts to find a treatment as much as possible and motivate patients to participate in natural history studies. To understand what is going on and to know what is needed, LAMA2-Europe will participate in the regular online meetings, which will be organised as a spin-off of this workshop. A request of the patient organizations towards clinicians and researchers was to be in patient-directed lectures more aware of the impact, which some statements might have on the patients and parents and to frame this in a way that it can be judged accurately by them. Also, harmonisation of the terminology used would be very helpful and prevent confusion. In scientific lectures, which patient organizations may also attend, this cannot always be fulfilled, so a disclaimer should be added, making these organization aware of these pitfalls.

- Clinical history/trial readiness of LAMA2-MD

Natural history studies are going on in France, Spain, the Netherlands, Switzerland, Italy, Brazil, and the US. This covers already more than 350 patients, including retrospective data. The ambition is to extend natural history studies to additional countries, like the UK, Portugal, New-Zealand, Australia, and Canada. The next steps will be to harmonise the data collection and define a minimal dataset, which will be collected for all patients. This minimal dataset could include the CHOP intend for patients <2 years, the MFM32 for patients >2 years (motor functions, range of motion (ranges <90°/>90°) of elbow and knee joints, rigid neck, adherence to standards of care, passive range of motion (PROM) and photogrammetry for monitoring of respiratory function). A definite agreement on the harmonisation and minimal dataset will be established in regular online meetings, that will be scheduled as follow-up of the workshop. Prospective and additional specialised investigations in subsets of patients will be shared.

- Therapy development for LAMA2-MD

The research groups working on therapy development agreed to strengthen their collaboration. First, regular research meetings will be scheduled to monitor and discuss progress in the different therapeutic strategies, which are being investigated. Next, an inventory will be made of available reagents, SOPs, antibodies, RNA, DNA, proteins, biomarkers, biological samples, tissue and animal models, patient tissues and cell lines. A Flox LAMA2 mouse model will be generated by the group of Markus Rüegg. Everything will be made available on a collaborative basis for

the research groups involved. Finally, funding opportunities will be jointly explored for financing research projects, but also for scientific meetings and workshops.

### Declaration of generative AI and AI-assisted technologies in the writing process

The authors did not use AI or AI-assisted technologies in the writing process.

### Declaration of competing interest

None

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**Hubert Smeets:** Funding acquisition, Supervision, Writing – original draft, Writing – review & editing. **Bram Verbrugge:** Funding acquisition, Project administration. **Xavier Bulbena:** Funding acquisition, Project administration. **Liliya Hristova:** Writing – original draft, Writing – review & editing. **Julia Vogt:** Writing – original draft, Writing – review & editing. **Isabelle van Beckhoven:** Writing – original draft, Writing – review & editing.

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