MDC1A: the road to therapy

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In the weekend from Friday the 15th to Sunday the 17th of November in 2019, we held the first international conference on the rare, inheritable muscular dystrophy MDC1A (Merosin Deficient Congenital muscular dystrophy type 1a). The Friday and Sunday of the conference were only accessible to experts. On Saturday the 16th patients, their parents and caregivers had the opportunity to speak to these international experts during round table sessions.

This interaction is important, because MDC1A is such a rare disease that one single physician does not exactly know how the disease progresses, nor how to measure the effects of treatments reliably, based solely on his own patients. All experts together do have this knowledge. The optimal treatment options, maintaining functions and quality of life, and the attainability of optimal care for MDC1A patients have all been topics of discussion.

The exchange of experiences was important as well. These were intense conversations that were greatly appreciated by all participants, which should lead to a higher standard of care and better treatment options for these patients. A number of experts gave presentations that were suitable for the general public. These presentations are further explained in this article/text/down here.

Presentation by Dr. Reghan Foley: “The clinical spectrum of MDC1A”

Dr. Reghan Foley received her BSLA degree and MD from Georgetown University, USA and is specialised in pediatric neuromuscular diseases. From 2010 to 2013, Dr. Foley was a Clinical Research Fellow at UCL Institute of Child Health and Great Ormond Street Hospital for Children in London. In 2014, she became an Investigator at the Neuromuscular and Neurogenic Disorders of Childhood Section of the National Institutes of Health. Dr. Foley’s research focuses on congenital muscular dystrophies and, in particular, on co-ordinating an international collaboration to study natural history. The main goal of her research is to promote progress towards clinical trials.

MDC1A stands for Merosin Deficient Congenital muscular dystrophy type 1a. The disease expresses itself in many different ways and the prognosis differs greatly among patients. Dr. Reghan Foley makes a distinction between a complete merosin deficiency, where the protein merosin (nowadays often called lamimin alpha 2) is not present in the body, and a partial merosin deficiency, witha decreased amount of merosin. Sincethe onset of symptoms with a partial merosin deficiency is at a much later age, the term LAMA2-related disease is used instead of MDC1A. This term encompasses both forms of merosin deficiency as.

A complete merosin deficiency is characterised by the following symptoms:

- Low muscle tone
- Inability to walk without mobility aids
- Joint contractures that can worsen in severity
- Difficulty breathing
- Mild reduction in function of the nerves supplying the muscles
- Brain abnormalities on MRI scans
- Epileptic attacks (in 30% of patients)

A partial merosin deficiency is characterised by much milder symptoms. Patients with a partial merosin deficiency can usually walk independently.

Dr Reghan Foley also presented the results of a phase I clinical study (safety trial) on a medicine called the Omigapil, that could possibly alleviate the symptoms of MDC1A. The Omigapil prevents cell death of muscle cells through apoptosis. A study on mice with MDC1A showed that the Omigapil reduced cell death, weigth loss and deformation of the skeleton, making it the mice move around more easily and live longer. In 2018 the safety of the Omigapil was tested in the USA on a group of Congenital Muscular Dystrophy (CMD) patients with ages ranging from 5-17. This research phase has been concluded, and it has been established that the Omigapil is safe for CMD patients. The next phase, where the effectivity of the medicine is tested, has currently been put on hold. The company that is developing the drug, Santhera, sees more potential in genetic therapy. This therapy can be applied to very young patients, which is why Santhera financially supports a natural history study on patients younger than five years old. On request of Dr. Foley, Santhera will continue research on the Omigapil, since it could be an effective adjuvant therapy. Further research will point out whether the Omigapil has a positive effect on the symptoms and progression of MDC1A in patients.
For more information of the safety study on the Omigapil and frequently asked questions about this medicine, see appendix 1 and 2.

Presentation by Dr. Hemant Sawnani: “Respiratory insufficiency (what, why, how)

Dr. Hemant Sawnani is trained in Paediatric Pulmonology and Paediatric Sleep Medicine. He is currently an Associate Professor at the Division of Pulmonary Medicine at Cincinnati Children’s Hospital Medical Center, USA.
Dr. Sawnani’s academic interest has focus on paediatric neuromuscular diseases and their impacts on sleep and breathing and chest wall architecture. MDC1A is one of those diseases.

Patients with MDC1A generally have difficulty breathing. This is because the muscles are weakened and stiff, which makes it harder for the chest to expand and for the diaphragm to move up and down. The tidal volume, the volume that is breathed in during each breath, becomes smaller because of this. It is important that the tidal volume remains large enough to facilitate adequate gas exchange of oxygen and CO_2 between the lungs and the blood. It also becomes more difficult to cough up mucus from the lungs. This can not only lead to shortness of breath and fatigue, but also to pneumonia, which can be fatal particularly in young children. That is why it is important to regularly perform a respiratory function test. Due to the fact that patients lay on their back at night, it becomes even more difficult to breathe at night. Whether there are breathing problems at night can be found out with a sleeping study.

There are several therapies that have been developed that can help MDC1A patients to reduce their respiratory complaints. One of such therapies, is called Couch Assist Therapy. This therapy makes use of a machine that makes patients cough by first inflating, and then rapidly deflating their lungs. This simulates a cough, loosening mucus from the lungs and making it easier to breathe. This process is repeated several times until the airway has been cleared. Another therapy is called hyperinflation. This entails inflating the lungs several times. This stretches the respiratory muscles, keeping them supple and preventing stiffening.

Another method is called Positive Airway Pressure Therapy (PAP). Here, a positive pressure is delivered over the airways, making it easier to breathe. There are two variants; one that continuously gives a positive pressure and one that changes between two different pressures, simulating breathing (BiPAP). This therapy can be used at night to support breathing during sleep.

These therapies help to reduce symptoms like shortness of breath and cause normal concentrations of oxygen and CO_2 in the blood. This leads to an improvement of sleep quality, quality of life and functioning in daily life. Furthermore, it is important to prevent irreversible loss of function, because this will improve the results of future therapies for MDC1A.

Presentation by Dr. Anna Sarkozy: ‘’Genotype-phenotype correlations in MDC1A and CMD’’

Dr. Anna Sarkozy studied medicine at La Sapienza University in Rome, Italy, where she also completed her higher medical training and PhD in clinical genetics. From 2008 until 2014 she worked in the Newcastle Muscle Centre, NSCT Service for rare Neuromuscular Disorders as a specialty doctor in Neuromuscular Genetics. In 2014, Dr. Sarkozy joined the Dubowitz Neuromuscular Centre where she currently works as a consultant in Neuromuscular Diseases. Dr. Sarkozy’s research interests lie in the identification of genes, clinical characterisation of rare neuromuscular phenotypes, the natural history of the diseases as well as genotype/phenotype correlations, in particular for congenital myopathies and muscular dystrophies like MDC1A.

Dr. Anna Sarkozy speaks of so-called genotype-phenotype correlations. These are connections between a specific change in a gene (genotype) and the observed effect in a patient (phenotype). In other words: what consequences (like muscle weakness, breathing problems etc.) are occurring in patients due to the genetic change (MDC1A). Therefore, genotype-phenotype correlations can be used to quickly form a genetic diagnosis, and figure out what causes a disease. They are also important to predict the prognosis of a patient and select the ideal treatment. Based on the genotype, it can be predicted how the disease will manifest in carriers of a specific genetic defect.

There are more than 20 different congenital muscular dystrophies, one of which is MDC1A. The genetic cause of MDC1A was found in 1994, namely a mutation in the LAMA2 gene, which codes for the protein laminin alpha 2, also known as merosin. Based on epidemiologic data, 30 per cent of CMD patients have MDC1A. Most of these patients have a complete merosin deficiency, in which no laminin alpha 2 is formed. Two to three per cent has the milder partial merosin deficiency, in which laminin alpha 2 is only partially absent. This type is not referred to as MDC1A, but as LAMA2-Related Muscular Dystrophy. It is important for a correct prognosis and an adequate treatment to know which mutations lead to the mild and severe form. Insight into the mild form, and how much laminin alpha 2 is present, is important for developing therapies that are aimed towards restoring the production of laminin alpha 2.

Presentation by Prof. Dr. Madeleine Durbeej: “Therapeutic strategies for MDC1A”

Prof. Dr. Madeleine Durbeej leads the Muscle Biology unit at Lund University, Sweden, which researches congenital muscular dystrophies, among which MDC1A. Over the years, her team has been able to identify the affected genes and proteins of the disease using various approaches. Based on this information, the unit has explored different genetic and pharmacological interventions to improve the well-being of the patients.

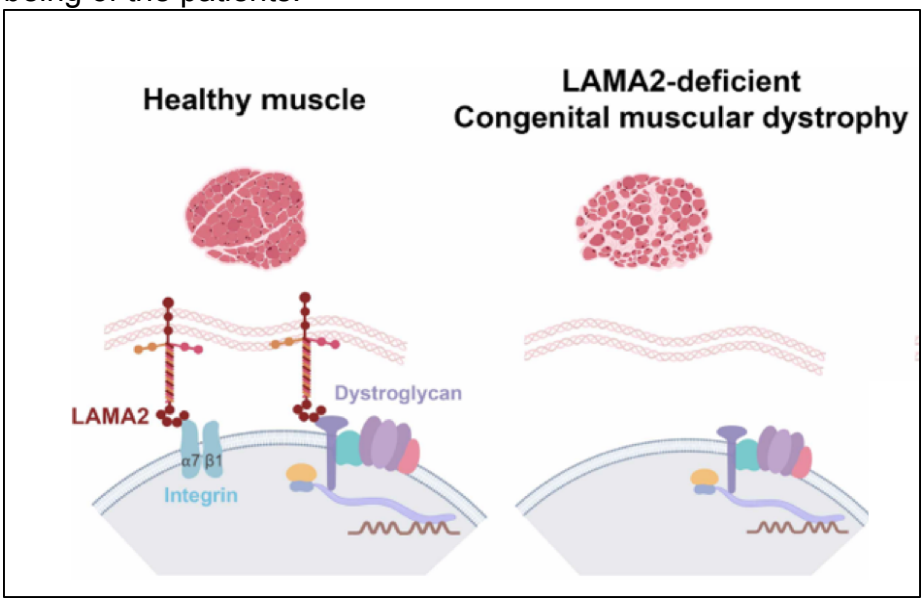


Figure 1: Healthy muscle compared to muscle of an MDC1A patient
On the left, laminin alpha 2, which is part of the protein complex laminin-411, anchors the healthy muscle to the basement membrane. On the right, in the muscle of an MDC1A patient, laminin alpha 2 is not present. Due to lack of anchorage of the muscle to the basement membrane, the muscle wastes away. For a more elaborate description, we refer you to the source of this image, an article written by Dwi Kemaladewi, whose presentation is discussed later.[1] (see references down below)

The cause of MDC1A is a defect in the LAMA2 gene, which codes for laminin alpha 2, or merosin. Laminin alpha 2 is part of a protein complex called laminin-411, which anchors the muscle to the basement membrane, which surrounds the muscle. Due to a mistake in the LAMA2 gene, laminin alpha 2 is partially or completely absent, which leads to insufficient anchorage to the basement membrane. The absence of this protein has previously been referred to as a ‘partial’ or ‘complete deficiency’. This leads to an increase of muscle weakness and eventually to muscle wasting. A visual representation of this process is displayed in figure 1.

An important therapeutics strategy is aimed at the recovery of the anchorage of the muscle to the basement membrane. This could be done by correcting the defect in the LAMA2 gene, or by activating the expression of a different laminin that can take over the function of laminin alpha 2. Another possibility are so-called linker proteins, proteins that facilitate anchorage of laminin-411 to the basement membrane instead of laminin alpha 2.
Both these methods have been tested in mice with promising results, like a decrease in muscle breakdown and recovery of muscle function. This shows the importance of good MDC1A mouse models. Therapies other than these two, are also tested on these mouse models (see figure 2). It is important to note that the anatomy and physiology of mice differ from those of humans, so it is uncertain whether the therapies will be as successful in humans. This will eventually be determined in clinical studies with MDC1A patients. Because MDC1A is a complex disease, a combination of therapies will be the most effective, according to Prof. Dr. Durbeej.

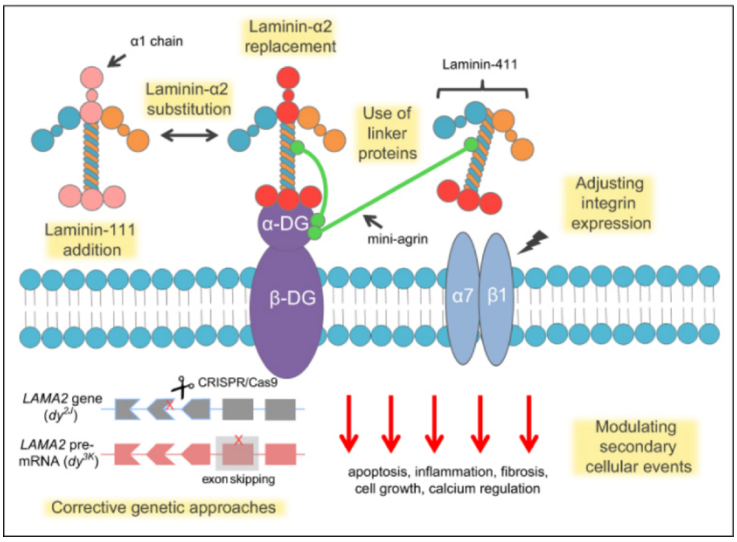


Figure 2: overview of possible treatments for MDC1A.
An overview of possible treatments to restore the anchorage of the muscle to the basement membrane, like repairing laminin alpha 2, adding a comparable protein that can take over the function of laminin alpha 2 or adding linker proteins such as mini-aggrin. Another possible therapy that is discussed later, is CRISPR/Cas9. For a more elaborate description, we refer you to the source of this image. [2] (see references down below)

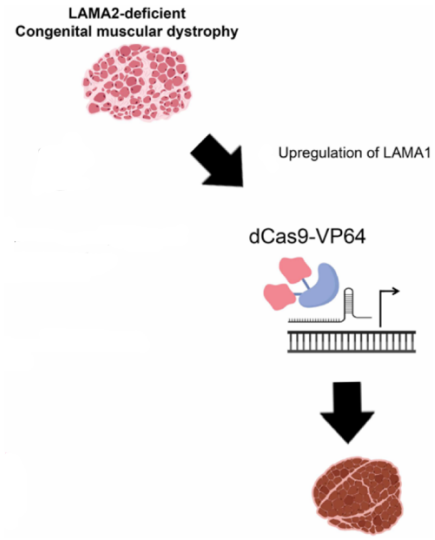
Presentation by Dr. Dwi Kemaladewi: ‘’MDC1A: The road to therapy’’

After completing a Bachelor in Life Sciences at the Hogeschool van Arnhem and Nijmegen in the Netherlands, Dr. Kemaladewi received her PhD in October 2012 from Leiden University, the Netherlands. From 2012 until 2018 she was a Research Associate at the Hospital for Sick Children in Toronto, Canada led by Prof. Dr. Ronald Cohn. Currently she is an Assistant Professor in the Department of Pediatrics at the University of Pittsburgh, USA. Dr. Kemaladewi is specialised in the development of genetic therapy, with a focus on neuromuscular disorders. Her research focuses on the underlying molecular mechanisms of MDC1A to study and develop therapies.

Dr. Kemaladewi has developed two strategies for the treatment of MDC1A and tested them on mice with MDC1A. The first strategy makes use of CRISPR/Cas9 to correct the mistake is the LAMA2 gene. CRISPR/Cas9 is brought into the mouse via a virus. After the correction of the gene, the mouse’s symptoms clinically improve. Many MDC1A patients have the same type of mutation, which makes this therapy applicable to a portion of patients. However, since different patients have different mutations, a specific CRISPR/Cas9 protein would have to be developed for each of them, which would require a lot of work. See figure 2 for an overview of treatment strategies.

The second treatment strategy work independently from the LAMA2 defect, which makes it applicable to all MDC1A patients. This strategy also uses CRISPR/Cas9, but instead of correcting LAMA2, the LAMA1 gene is activated. LAMA1 is a gene that encodes the protein laminin alpha 1, which has the same function as laminin alpha 2, but is usually only active during embryonic development. Laminin alpha 1 is then later replaced by laminin alpha 2. As it turns out, laminin alpha 1 is perfectly capable of taking over the function of laminin alpha 2. Dr. Kemaladewi was able to activate the LAMA1 gene in mice with MDC1A, which lead to big clinical improvement. She also tested this approach in muscle cells of MDC1A patients and demonstrated that the LAMA1 gene becomes active again.

To go from cells and mice models to humans, more research is needed on two important aspects. First, more research is needed to know more about the sasfety and the molecular mechanisms behind dCRISPR-LAMA1. Secondly, more research is needed on the feasibility and the safety of gene therapy (can all muscles be reached with gene therapy, is this safe, what is the correct dosage, how long will the effects last, et cetera).



Figuur 3: CRISPR/Cas9
Instead of correcting LAMA2, a gene called LAMA1 is activated, which activates the production of laminin alpha 1. This protein has the same function as laminin alpha 2, the affected protein in MDC1A, but is only active during embryonic development. For a more elaborate description, we refer you to the source of this image. [1] (see references down below)

Presentation by Dr. Giulio Cossu: “Stem cel therapy in CMD”

Prof. Dr. G. Cossu received his MD degree from the University of Rome in 1977. He trained as a post-doctoral at University of Pennsylvania, and then became Associate Professor at the University of Rome. In 2000 he became Director of the Division of Regenerative Medicine at San Raffaele in Milan. In 2012 he moved as Professor of Human Stem Cell Biology to University College London and in 2013 to the University of Manchester. Dr. Giulio Cossu discovered the possibility to restore damaged muscle tissue by using muscle stem cells. This therapy was proven effective in mice and dogs models with muscular dystrophies. He has also done research on the use of stem cell therapy in patients with Duchenne muscular dystrophy.

Epidermolysis bullosa (EB) is a skin disease caused by defects in laminins, like laminin beta 3, that anchor the outer layer of the skin to the deeper layers. A defect in these laminins will cause the skin to come loose, which causes wounds and blisters. In 2015 in Germany a young boy with EB, caused by a mutation of the LAMAB3 gene, was successfully treated. He had lost a large portion of his skin. A biopsy of an intact part of skin was taken, and his skin cells were cultivated. The genetic mistake in these cells was corrected using gene therapy. The cells were then transplanted back to the patient. In 2016, the boy was discharged from the hospital with strong, healthy skin.

A similar approach is possible for the treatment of muscular dystrophies, like MDC1A, where diseased muscle cells are corrected using CRISPR/Cas9. The corrected cells are then placed back. Unfortunately, there is one problem. This therapy is easy to apply to tissues like the skin or the blood, because the cells can be transplanted directly to the right location. In muscles, this is more complicated; many injections would be needed to deliver the cells to all the muscles in the body.
This problem can be solved by using a certain type of stem cells called mesoangioblasts. These stem cells are isolated from a muscle biopsy from the patient, after which they are corrected and multiplied. The mesoangioblasts are then injected into the blood and travel to the muscles, where they form new muscle fibers by fusing with existing muscle fibers. If the treatment is effective enough, one single treatment could be enough to treat muscular dystrophies. It is also possible to use stem cells from a healthy donor, but this has the drawback that transplant rejection can take place, and immunosuppressive drugs would make this therapy less effective.

One of the challenges of this therapy is the large amount of stem cells that is needed to treat all of the muscles. Moreover, only a fraction of the injected cells actually reach muscle tissue. Furthermore, the muscles need to be inflamed, because this attracts the stem cells towards the muscles. It has been demonstrated that stem cell therapy with donor mesoangioblasts is effective in mice and dogs for several different muscular dystrophies. Treatment with healthy donor cells has been tested in patients with Duchenne muscular dystrophy. The treatment seemed relatively safe, but the effectivity was limited. A possible explanation for this, is that the treatment was not performed optimally. Future research is aimed at improving genetic correction so that

patients can be treated with their own stem cells, and to make transplantation to hard-to-reach muscles possible, like the back muscles and the diaphragm. Hopefully, an effective therapy will be developed.

Round table sessions

During the round table sessions, patients, relatives and physicians had the opportunity to personally speak with the experts and ask questions. There was also room for patients, relatives and physicians to interact with each other, and share personal experiences regarding MDC1A. The English presentation were translated to Dutch by the students that were present, for anyone who had the need.

Conclusion

From this conference it has become clear that a lot of research on muscular dystrophies, like MDC1A, is taking place. More is being discovered about the underlying mechanisms and expressions of MDC1A. A lot of research is being done on possible new treatments as well, among which are gene therapy, stem cell therapy and the Omigapil. Besides that, there is a lot of attention for maintaining function in patients, like supporting breathing. Due to the rarity of MDC1A, not a lot is known about the natural history of the disease. During the conference, concrete agreements have been made to internationally do retrospective and prospective natural history studies on MDC1A patients, with the help of CureCMD. With this, parameters that show the effectiveness of new treatments can be established. Furthermore, it turned out that not all patients received optimal care. It has been agreed to translate the international care guidelines to Dutch, so that patients and their caregivers can share this with their phycisians. In addition, Dutch patients will be centrally managed by Dr. Nicol Voermans (Radboud UMC). Finally this MDC1A conference will be repeated (bi)yearly to strengthen collaboration in the road to therapy.

Appendix 1 CALLISTO Clinical Study Results

Appendix 2 CALLISTO Clinical Study Frequently Asked Questions

References Figures

1. Kemaladewi DU, Cohn RD. Development of therapeutic genome engineering in laminin-α2-deficient congenital muscular dystrophy. Emerging Topics in Life Sciences. 2019;3(1):11-8.
2. Nguyen Q, Lim KRQ, Yokota T. Current understanding and treatment of cardiac and skeletal muscle pathology in laminin-alpha2 chain-deficient congenital muscular dystrophy. Appl Clin Genet. 2019;12:113-30.