2019 Cure CMD Scientific & Family Meeting

July 25-28, 2019, Chicago, USA

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Cure CMD, the largest congenital muscular dystrophies patient association in the United States, brought together, once again, this time in Chicago, researchers and families to meet and connect, as well as to share news about ongoing research on treatments and cures for these rare diseases.

Noelia Foundation also attended this reference event to learn first-hand how Collagen-VI congenital muscular dystrophy (Col6-CMD) research projects are progressing and to ensure our families are fully up to date with the latest trends in therapy and patient management. On this occasion, in addition to Cure CMD, two other associations dedicated to the promotion of research and to raising awareness about muscular dystrophies, Building Strength (nemaline myopathy) and Team Titin (titin deficiency), contributed to organizing the venue that brought together over 500 participants, 300 of which were part of the Collagen-VI community.

In this exceptional framework, Noelia Foundation had the privilege of presenting its first poster communication in a conference meeting of the highest social and scientific level, in the field of congenital muscular dystrophies. The poster showcased our work and achievements so far, as well as the projects that are currently being funded in our fight against Col6-CMD.

Diagnosis update

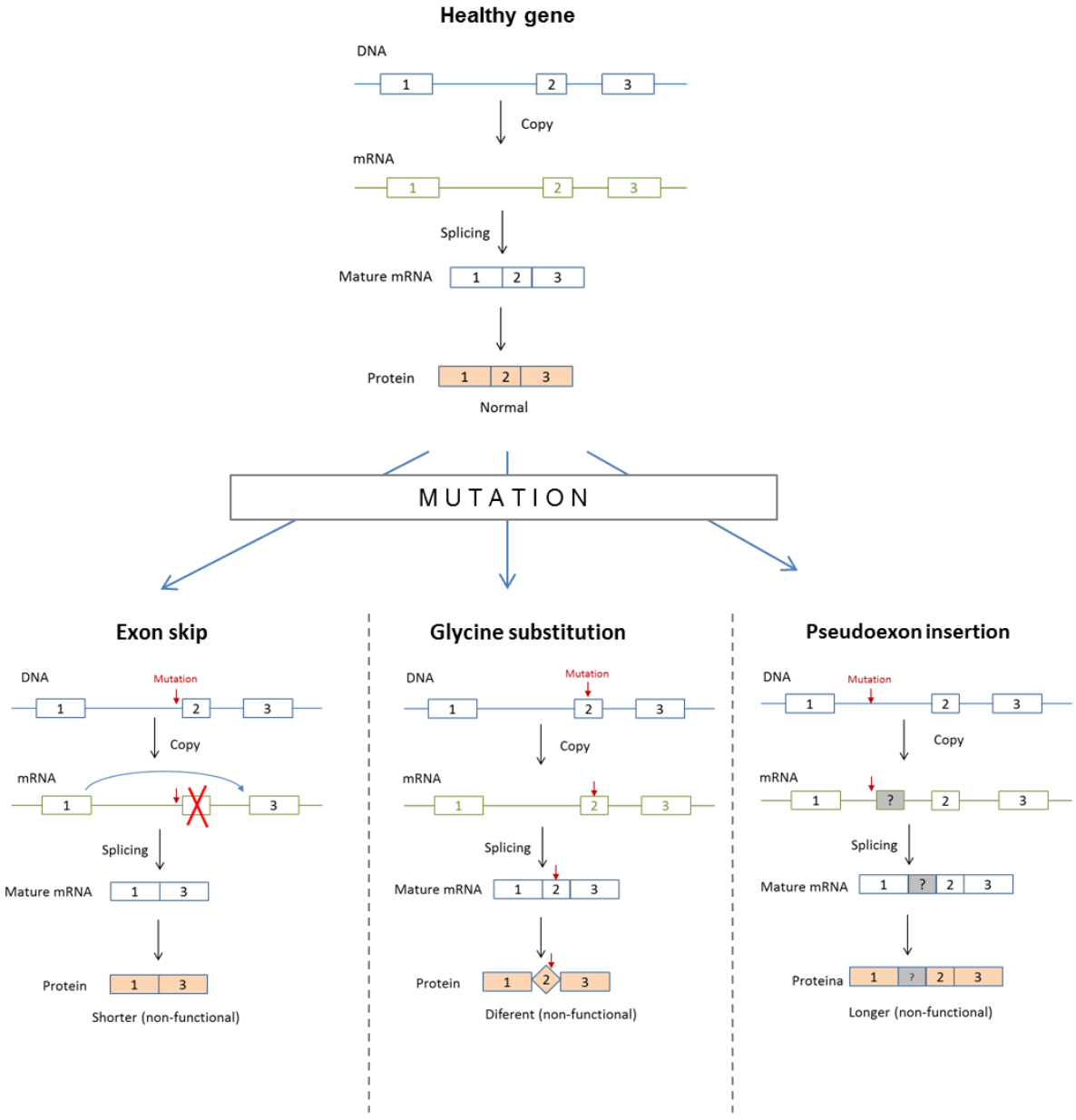
Unfortunately, there are still no specific biological markers that can provide a simple, fast and certain way to diagnose patients, nor reliable quantitative indicators to effectively and objectively validate their evolution. Right now, clinicians still have to rely on traditional motor function scales and spirometric measures.

For this reason, all participants in the clinical workshops insisted in the absolute need to address diagnosis of any case suspicious of being CMD through genetic testing. In this way, as soon as the underlying cause is identified, appropriate therapeutic and prophylactic measures can be applied to address specific needs. Also, being certain of their specific condition, patients may be tagged as potential candidates to the application of new therapies. A wrong diagnosis, on the other hand, may cause the patient to stop looking for the cause of his or her condition and remain subject to an inappropriate therapeutic program and excluded from any clinical advances that may arise.

Additionally, genetic testing can accurately identify the mutated sequence, which, in turn, may help identify the actual effect that such change in the gene causes in the reading process and the subsequent protein synthesis.

So far, three mechanisms have been identified by which a mutation results in a dysfunctional protein and, therefore, causes the clinical manifestations (see Fig. 1). Knowing the mechanism involved is key to determine which type of gene therapy may be applied, if and when it becomes available.

Fig. 1. Mechanisms and effect of the three types of dominant negative mutation in collagen VI genes



Location	In an intron, close to the intrón-exon joining point	In an exon (coding part of the gene)	In an intron, away from the flanking exons
Effect on mRNA	Reading error that results in one exon (coding fragment) being excluded.	Modifies the protein sequence. One aminoacid (glycine) is replaced by a different one*.	A new exon appears, which is not present in the wild-type protein
Effect on the Col6 protein	Shorter protein. Lacks a necessary fragment	Protein with the correct length but with a modified aminoacid sequence, which changes its 3D structure	Longer protein. Has an extra part that distorts its 3D structure
Biologic effect	In all cases, the altered protein is capable of assembling to the normal chains, but produces a dysfunctional collagen complex		

* All chains of Col6 (A1, A2 and A3) have a central region that contains a repeated sequence of aminoacids, which is always composed of a Glycine (Gly) followed by two other aminoacids that vary along the repeats (e.i., Gly-X-Y-Gly-X-Y-Gly-X-Y-…). This region is responsible for the assembly with the other Col6 chains. If a mutation modifies one of the glycines, the central region of the protein, despite having the right size and being able to assemble, does so incorrectly, distorting the shape of the final collagen complex and altering its biological functionality.

Update on the development of CMD therapies

As we have mentioned in previous occasions, the treatment of this type of diseases is, as of today, only palliative, since there is no known cure for the disease. However, there are several approaches aimed at identifying curative treatments that are currently under way. Each of them seeks to address a different stage of the pathological process, from the mutation in the DNA itself to the final effect of muscle tissue destruction. Of course, the closer we get to the origin of the problem (that is, the DNA), the more likely we are to achieve a true therapeutic effect that goes beyond alleviating the symptoms.

The existing mouse models, although they do not show particularly severe clinical conditions, are very representative of the manifestations of the disease in humans, so they constitute a very good platform on which to test and develop new therapies. This tool is currently being used in several laboratories in the US and Europe, with a number of mouse models specifically designed for the study of Col6-CMD.

However, sooner or later, it will be necessary to evidence both safety and efficacy of these therapies in humans before they can be widely used in clinical practice. And clinical studies in rare diseases are difficult to carry out because of the limited availability of patients and, also, because most patients are children or youngsters, a group in which the ethical factor is particularly relevant making study design even more complex. In addition, since there is no clear understanding of the development of the disease at the molecular level or which are the most appropriate biochemical markers to assess its evolution, it is difficult to design studies that clearly show the effect of new treatments.

In this regard, there is a general consensus that knowing the natural history of the disease is essential. If we know how untreated patients evolve (given that there is no treatment yet), we can see how this evolution changes when a certain therapy is applied. Additionally, understanding the natural history contributes to produce more accurate clinical diagnoses, provides a better ability to predict the evolution of patients, and allows conducting neonatal screenings to identify early signs that may help discover windows of opportunity to implant therapies at early stages.

For this reason, one of the most important and ambitious projects of the Noelia Foundation is the Natural History Initiative that is being conducted at the San Joan de Déu Hospital in Barcelona in coordination with several hospitals in Spain and the US, and follows 120 pediatric patients to assess in detail, as objectively as possible, how they evolve. The data collected in real time will provide a very solid benchmark for future clinical trials. This study will be the first ever in Col6-CMD patients so far, and will add to those already existing for other congenital muscular dystrophies such as laminopathies, merosin deficiency, titinopathies or nemalinic myopathy.

Pharmacological therapy: Omigapil and Santhera's Callisto trial

In the pharmacological field, some drugs are emerging that are intended to alleviate the symptoms caused by collagen dysfunction. Omigapil is the one that has advanced most down its development pathway, having reached already the clinical testing phase. This drug is an inhibitor of apoptosis (the natural process of cell destruction), which is abnormally exacerbated in muscle cells of Col6-CMD patients as a result of the extracellular matrix dysfunction, and is the cause of the characteristic progressive muscle deterioration.

At the symposium, representatives of Santhera, the Swiss company leading the development of omigapil, along with the principal investigator of the study, Dr. A. Rhegan Foley, from the National Institutes of Health in the US, presented the results of the Callisto trial. This study aimed to identify the most appropriate dose of omigapil in children and adolescents. On the basis of these results, a new study will be designed to determine the efficacy of the product in the treatment of congenital muscular dystrophies due to merosin

deficiency and collagen-VI deficiency, the two diseases that, as of today, are considered susceptible to treatment with the drug.

The final report of the Callisto trial will be published shortly, at which point both Cure CMD and Noelia Foundation will share it on their respective websites, for those interested. In summary, the results show that:

1) The most appropriate dose is 0.06 mg / kg
2) No therapeutic effects have been observed in either of the two patient groups (10 patients with merosin deficiency and 10 patients with Col6 deficiency, of various ages). However, the duration of the trial was only 12 weeks and, in any case, the drug is not expected to have any effect before several months of treatment.
3) Santhera values the product as safe and well tolerated. Although a total of 185 adverse effects were recorded during the 12 weeks of the trial, only two of them were concluded to be related to the drug (upset stomach and low blood pressure).

In view of these results, the scientific committee that supervised the Callisto trial (TreatNMD Advisory Committee on Therapeutics or TACT) issued, among others, the following recommendations:

- Patients with merosin deficiency and those with Col6-CMD should be considered separately. Assessing the effect of omigapil on both types of muscular dystrophy together may distort results and conclusions.
- More data is needed on the dose-effect relationship in animal models in general and, in particular, additional efficacy data in a mouse model of Col6-CMD.
- Specific biomarkers are needed to precisely assessment the effect of the drug, although muscle function scales are considered to be an adequate measure at this point. For the moment, the use of variables derived from magnetic resonance imaging or muscle biopsies is ruled out due to their current limited precision.

Based on this, the evolution of the project is foreseen in the following terms:

- Thanks to the fact that trial patient's urine and blood samples were collected before, during, and after the administration of the drug, a screening will be carried out in search for biomarkers relevant to the biochemical processes of muscular dystrophy and that may show changes as a result of the treatment.
- Regarding the second critical point, the need for additional preclinical data in mice models with Col6-CMD, Dr. Jodi Wolff, Head of Patient Relations at Santhera, informed us that, thanks to the opportunity offered by the Chicago venue, she managed to coordinate resources from several laboratories to conduct these tests in the coming months.

Therefore, although the first clinical study with omigapil has been successfully completed, more data, both preclinical and clinical, is still needed before it can be said that the drug is a potential new therapy for our Col6-CMD patients.

In addition to omigapil, Dr. Bönnemann mentioned that other drugs are being assessed. Some products that are already marketed for different conditions show pharmacological effects (either primary or secondary) that may be useful in the treatment of Col6-CMD. However, there is only preliminary data, so neither can be considered yet as real therapeutic alternatives.

That said, all participants in the symposium agree that the dysfunction of the extracellular matrix causes progressive degradation of muscle tissue. It is even suggested that the mechanism that causes such muscular dysfunction may change throughout its progression, so, pharmacological interventions may vary along its course.

The solution, however, is at a point upstream from all these metabolic phenomena, that is, in the defective expression of the gene, so gene therapies are the ones that, in principle, offer the greatest options for success.

Gene therapy: antisense oligonucleotides

The modification of DNA to correct a mutation is a complex process, not devoid of risks and, as of today, still too immature to become a widely used therapy in patients. Therefore, the approach that is emerging as most promising in terms of its viability and its chances of reaching the clinical stage in a reasonable time, is the prevention or correction of the defective gene expression. This can be achieved through several techniques, but the most advanced is the administration of antisense oligonucleotides (AO) specifically designed for each mutation.

Examples of this strategy are found in products such as Exondys 51 (eterlipsen), developed by the US company Sarepta Therapeutics and marketed since 2016 for Duchenne's muscular dystrophy, as well as two other products for amyotrophic lateral sclerosis (ALS). Although these are fairly early examples, which effectiveness is limited by the inherent characteristics of these diseases, they evidence the viability of these types of therapies both at the development level and in terms of their options of obtaining approval by health authorities before they become available to all patients.

In the particular case of Col6-CMD, as described above, there are three mechanisms by which a negative dominant mutation (the most common in Europe and America) can affect the collagen function at cell level. The therapeutic approach needed in each case will, therefore, depend on the underlying mechanism. All approaches, however, are based on the administration of antisense oligonucleotides (see Table 1). The two approaches, known as allele-specific silencing and pseudoexon skipping, are being developed by Dr. Bönnemann's team in the framework of two research projects co-funded by the Noelia Foundation.

Table 1. Gene therapies applicable according to the type of mutation in the Col6 genes

Mechanism of the mutation	**Exon skip**	**Glycine substitution**	**Pseudoexon insertion**
Therapeutic strategy	Mutated allele specific silencing		Prevent reading of the newly created exon (exon skipping technique)
Type of therapy	Antisense oligonucleotides that specifically bind mRNA and interfere in the splicing process: small interference RNAs (siRNA)		Antisense oligonucleotides that specifically bind the mutated part of the mRNA and mask the extra exon so it cannot be read
Expected result	Only the correct copy of the gene is expressed *		Normal collagen VI expression is restored

* This outcome has been described as non-pathological, despite the production of lower amounts of collagen.

Status of research projects on antisense oligonucleotide (AO) therapies

Gene Silencing Project

This project studies the effect of the administration of AO that interfere with the splicing (e.g., the maturation process) of the mutated mRNA. The results so far show that the *in vitro* administration of the therapeutic AO almost completely blocked the expression of the mutated allele in human fibroblast cultures. At the same time, the total production of collagen protein in the cells remained intact. In addition, the administration of AO restored the microscopic aspect of the extracellular matrix, which showed a structure identical to that of healthy tissue. The cellular model used in this project was cultured fibroblasts from a patient biopsy, with a dominant negative mutation of the COL6A1 gene that causes a glycine substitution.

Altogether, these results strongly suggest that the administration of AO may, indeed, constitute a viable option to revert the effects of the mutation.

Based on these results, the next step is the administration of these AO to mouse models, to determine how and to what extent the AO can reach the fibroblasts, which is where they actually need to exercise their effect, being the cells that produce the collagen for the extracellular matrix.

Pseudoexon skipping project

This project draws from the advances made in the research on therapies for Duchenne's. Products such as Exondys 51 have effectively been able to cause an exon skip and prevent the mutated sequence of the gene from being read. For Col6-CMD, specific AO were administered *in vitro* to fibroblast cultures from two patients, in an attempt to mask the new exon created by the mutation and prevent this segment from being read by the mRNA splicing machinery.

The results were presented by Dr. Carsten Bönnemann's team in the form of a brilliant intervention at the symposium by Dr. Veronique Bolduc and the poster entitled “A novel target for splice-modulating therapies: a common pseudoexon-inducing mutation that causes a severe collagen VI-related muscular dystrophy”.

The results show how this strategy was capable of correcting the reading error in the COL6A1 gene, and suppressed the expression of the mutant form of the protein. Consequently, collagen microfibrils were longer in treated cells and the extracellular matrix showed an improved structural aspect.

As in the Gene Silencing Project, the next step is to test the administration of the AO in animal models.

The big challenge: systemic administration

Having shown the ability of these gene therapies to reverse the genetic defect at the cellular level, the next step is to move to an *in vivo* environment. To do this, mouse models have been chosen as they have proved to be good tools for the study of new therapies. Currently, there are several published mouse models of Col6-CMD, some of which have been developed by Dr. Bönnemann's own team and others are in the process of creation in various laboratories around the world.

Whatever the type of mutation or genetic strategy, however, the biggest current challenge is to administer AO easily and effectively to patients. As promising as the results obtained in a cell culture plate are, the administration of intravenous AO, to this day, is not yet advanced enough to guarantee that the treatment will reach the muscle tissue adequately.

For certain pathologies, specifically engineered viruses can be used to deliver the AO. These viruses are modified so that, instead of causing a disease, they serve as mere vehicles for transporting the drug or, in this case, the AO. Once injected into the patient, the virus will migrate through the bloodstream to the target cells and deliver its payload (either the drug or the AO).

Unfortunately, although there are viruses approved by health authorities for muscle cell therapy, none of them works with fibroblasts, which are the cells we are interested as they are the ones responsible for producing the collagen for the extracellular matrix. However, this is a field where work is just beginning now and it is expected that sooner than later there will be vectors available.

While we wait for suitable vectors for fibroblasts, we must focus on other strategies to get the AO into the cells. In this regard, it has been shown that certain chemical modifications of the AO offer good results as an alternative to their encapsulation in a virus.

This challenge is common to all gene therapies based on the administration of AOs, whichever the mutation or the mechanism might be, and, therefore, is the greatest obstacle to the progression of research. For this reason, that is Noelia Foundation and Dr. Bönnemann's laboratory are going to focus all its efforts in the coming months with the aim to overcome this issue and move to test the therapy on animal models as soon as possible.

Trends in patient management

Given its degenerative nature, there is a clear consensus on the fact that, in all these pathologies, the success of potential therapies is expected to be all the greater, the more functional the patient's muscle is. Therefore, it is crucial to carry out any interventions that may be able to maintain muscle structure and function as preserved as possible.

The most widely acknowledged strategy, because it is available to everyone, is to preserve functionality and elasticity of muscles and tendons through physiotherapy, with the aim of delaying the degenerative process as much as possible, so that, when new therapies arise, the patient will have a sufficient base of healthy tissue to benefit from them.

Maintenance of the respiratory function

A brilliant panel of experts, from prestigious medical centers across the US, shared their experience and answered questions from the community of patients and families who attended the event:

- Dr. A. Reghan Foley. Pediatrician specialized in neuromuscular diseases. She is part of the clinical team of the Child Neuromuscular and Neurogenetic Diseases (NNDCS) Section of the National Institutes of Health, in Bethesda (Washington), and collaborates in several the research projects on Col6-CMD with Dr. C. Bönnemann.
- Dr. John Pascoe (specialist in pediatric pulmonology, Cincinnati Children's Hospital)
- Dr. MyMy Buu (professor and specialist clinician at the Pediatric Pulmonology and Cystic Fibrosis Clinic of Stanford University School of Medicine)
- Dr. Oren Kupfer (pediatric pulmonology specialist, Children's Hospital of Colorado)
- Dr. Hank Mayer (pulmonologist and Director of the Pulmonary Function Study Laboratory of Children's Hospital of Philadelphia)

The experts' panel members concurred on the importance of monitoring respiratory function in patients with muscular dystrophy, since the diaphragm, which job is to expand the lungs to inhale, is one of the muscles that suffer the most with the progression of the condition. Statistically, an annual loss of 3.5% of lung capacity can be expected in Ullrich patients, a 2.1% in intermediate phenotypes and a 1.2% in patients with Bethlem. This loss may be first observed in the patients (through clinical measures) between 7 and 12 years of age but, like many other parameters, it can vary significantly from one patient to another.

The main difficulty in managing the respiratory function of these patients lies in recognizing the absolute and fundamental need to keep their airways clear to facilitate ventilation, since lung function is the main determinant of the quality of life of patients with muscular dystrophies. Poor ventilation limits their ability to oxygenate the tissues and compromises sleep, at which time, respiratory movements become more difficult because of the horizontal position of the body.

The panel also points to sleep studies as an important element to monitor respiratory function, especially in children, and they insist that even small alterations of sleep patterns with no apparent relevance (abnormal tiredness, morning headaches, etc.) should raise the suspicion of potentially compromised ventilation during sleep.

This is where the views of pulmonologists and specialists in congenital muscular dystrophies come into contrast. The former determine the need for external ventilation aids based strictly on respiratory parameters (spirometry variables), while muscular dystrophies specialists consider that these are not precise enough to allow for an early detection of the need for intervention. For that reason, in the case of children and adolescents (up to 20 years of age), which undergo a continuous growth process and, therefore, their anatomical structure is permanently modified, repeated sleep studies over time are recommended as a means to detect potential decreases in respiratory capacity as early as possible. In addition, they suggest carrying these out including the assessment of CO2 levels, a very good indicator of the actual level of tissue oxygenation. In adults, sleep studies may be spaced out while effectively monitoring patients using a simple oxymeter.

Dr. Foley also insisted on the importance of respiratory physiotherapy, especially in patients in the critical age of 7 to 12. This can be done initially with a manual spirometer, such as Voldyne® 5000 or Triflow, which range of exercise is limited to the patient's own capacity, or with an Ambu®, which allows the caretaker to force inspiration beyond what the patient can achieves alone, thus increasing the range of the exercise.

When these are no longer sufficient to properly exercise respiratory muscles, mechanical devices can be used, always under proper medical supervision. For this, there are two main alternatives, which are the most commonly used: Cough-Assist® (Philips) and Alpha-300® (Air Liquide Medical). Although both fulfill their function of maximizing inspiratory movements, Dr. Foley is more in favor of Cough-Assist®. This device in particular, in addition to respiratory physiotherapy exercises when the device is in inspiratory mode, it provides an expiratory mode. When there is mucus production that needs to be cleared from the airways, the device forces the patient’s cough and facilitates the expulsion of the secretions before they can settle down deep in the lung where they may cause more serious complications.

At later stages, if the respiratory parameters suggest a decrease in lung capacity below a tolerable limit, and the risk of nocturnal respiratory failure arises, non-invasive night ventilation by means of a BiPAP may be considered. This device provides a night time respiratory support through a mask, by supplying oxygen and pressure when the patient is in a horizontal position and the diaphragm has to make an extra effort to expand the lungs.

As for the usual pharmacological treatments in pulmonology, the experts’ panel does not recommend the regular use of asthma medications such as inhalers (salbutamol and budesonide) or mucolytic drugs such as those used in patients with cystic fibrosis (e.g., nebulized hypertonic saline). These products may help solve a momentary crisis but should only be administered during an acute episode and not chronically. Inhalers dilate the bronchi and reduce air pressure, making it more difficult for the patient to clear the airways. Hypertonic solution stimulates mucus production, which is precisely what we want to avoid.

Shortly, Cure CMD will publish a guide of respiratory management in patients with muscular dystrophy which has been developed by a group of specialists, and that we will also share with families as soon as it becomes available.

Conclusions

The 2019 Chicago Symposium was a great success, both because of the high participation of medical specialists and families, as well as because of the extraordinary scientific level of the talks.

In addition, we witnessed the superb coordination and collaboration of all research groups, which is the keystone of an effective translation of the results presented into actual steps towards finding a cure.

Finally, we were pleased to learn about the excellent results achieved by the research groups devoted particularly to Col6-CMD because they give us hope and motivation to keep fighting to support research for a treatment that may help our children.

Noelia Foundation works day after day to raise funds to support research on Collagen-VI congenital muscular dystrophy, and these events strengthen our commitment and our firm belief that scientific breakthroughs will soon bear fruit.