COLLAGEN-VI CONGENITAL MUSCULAR DISTROPHY: CURRENT THERAPEUTIC APPROACHES

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Summary

Collagen-VI Congenital Muscular Dystrophy (COL6-CMD) is a rare degenerative disease of genetic origin, characterized by muscular weakness, for which there is currently no cure available. At present, numerous lines of research are aimed at finding a treatment for this and other similar genetic diseases, some of which can be a cause of death of the patient within the first years of life. The approaches that are being taken range from the administration of drugs that alleviate the symptoms, to gene therapy aimed at correcting the original defect in the DNA. In this article, we describe the main current lines of work for COL6-CMD, their development stage and the short and medium term perspectives they offer as real therapeutic alternatives.

Introduction: the origin of the disease

Collagen VI Congenital Muscular Dystrophy (COL6-CMD) is a rare degenerative disease (affecting less than 1 among 30,000 children) caused by a mutation in one of the three genes (COL6A1, COL6A2, COL6A3) involved in the synthesis of the collagen in skeletal muscle. It is characterized by muscle weakness, hyperlaxity in fingers, hands and feet, muscle contractures, and spinal rigidity.

Collagen (COL) is a molecule present throughout the body that is a key part of the extracellular matrix, a protein superstructure that surrounds the cells and provides structural and biochemical support. The function of the extracellular matrix is crucial for the proper function of any tissue. It provides the media through which cells communicate among them to carry out their function, multiply and, eventually, die and be replaced.

There are many types of collagen (so far 28 have been identified) each of which is mainly present in certain tissues and not in others: type-VI collagen (COL6), in particular, is present almost exclusively in skeletal muscle.

COL6 is a very complex structure, formed by three protein chains (A1, A2 and A3) that are intertwined to produce one single molecule of COL6. In turn, this molecule is assembled with other molecules of COL6 to make up a protein complex of 8 units, which is the functional form of COL6 that will be used by the cells to build the extracellular matrix.

However, if any one of the chains has a mutation that causes the strand to have an inadequate length or shape, the initial braiding of the three strands does not occur normally and the resulting protein complex lacks the necessary characteristics to build a fully functional extracellular matrix. This causes the appearance of the pathology, which causes a dysfunction of the muscle tissue (muscular dystrophy). The extracellular matrix built with defective collagen is not capable of carrying out its function, which has, among others, two fundamental effects: (a) muscle weakness, because a component of the tissue is not fully functional and (b) an increase in programmed cell death (apoptosis) of muscle cells, which is responsible for the degenerative nature of the disease.

[1] Dr. Carsten Bonnemann is child neurologist and Chief of the Neuromuscular and Neurogenetic Disorders of Childhood Section at the National Institute of Neurological Disorders and Stroke (NINDS, part of the National Institute of Health in Bethesda, Maryland, USA). Research in Dr. Bonnemann's laboratory revolves around molecular mechanisms underlying early onset muscle disease (congenital muscular dystrophies, congenital myopathies, and reducing body myopathy). The laboratory's goal is to identify the genetic and cellular mechanisms in these conditions in order to develop strategies for molecular-based treatments.

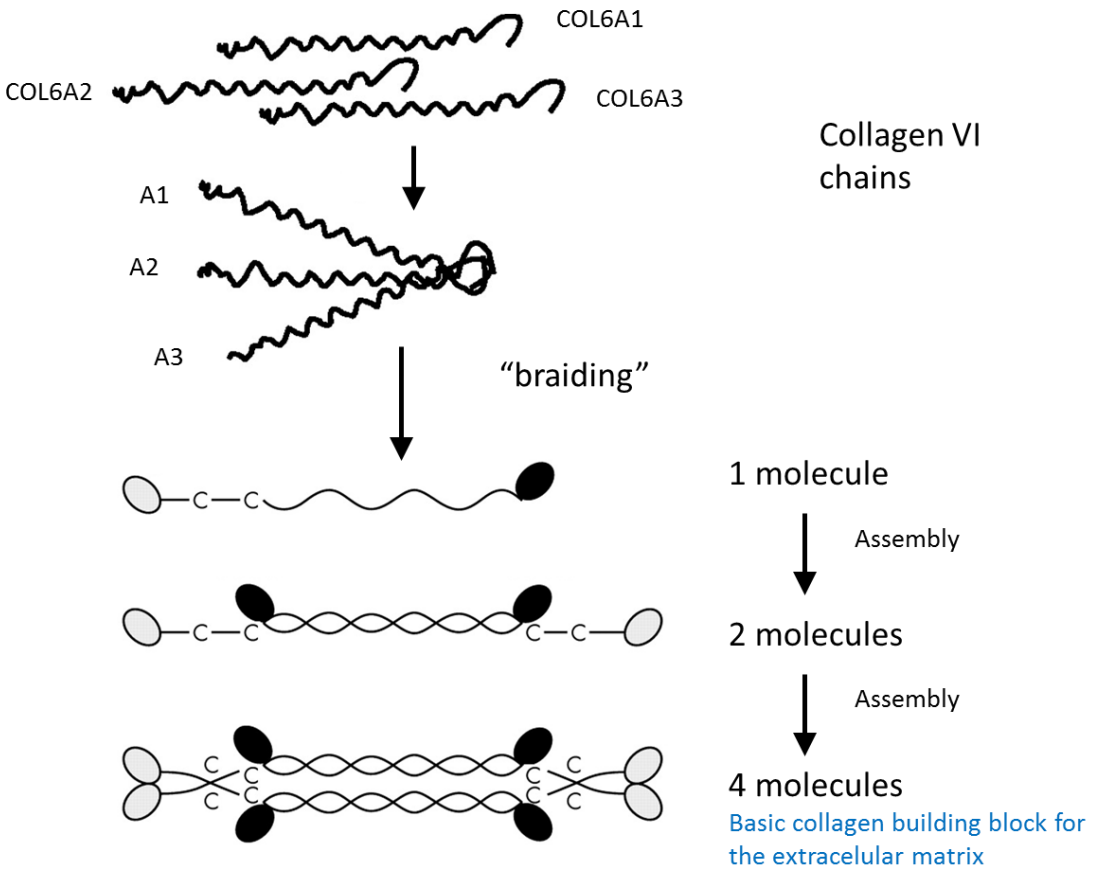


Fig. 1. Formation of the collagen VI complex under normal conditions

However, not all mutations cause the disease. There are mutations that make the resulting protein so short that it does not even assemble with the rest of the chains and is degraded as any other piece of waste of the metabolism. Because every human being has two copies of each gene (one from the father and one from the mother), the protein generated by the second copy of the gene, although present in a lesser amount, is sufficient to form a functional extracellular matrix and prevent the appearance of pathological symptoms. This has been proven in individuals that display a total absence of expression of one of the two copies of the gene, but did not show any manifestation of the disease.

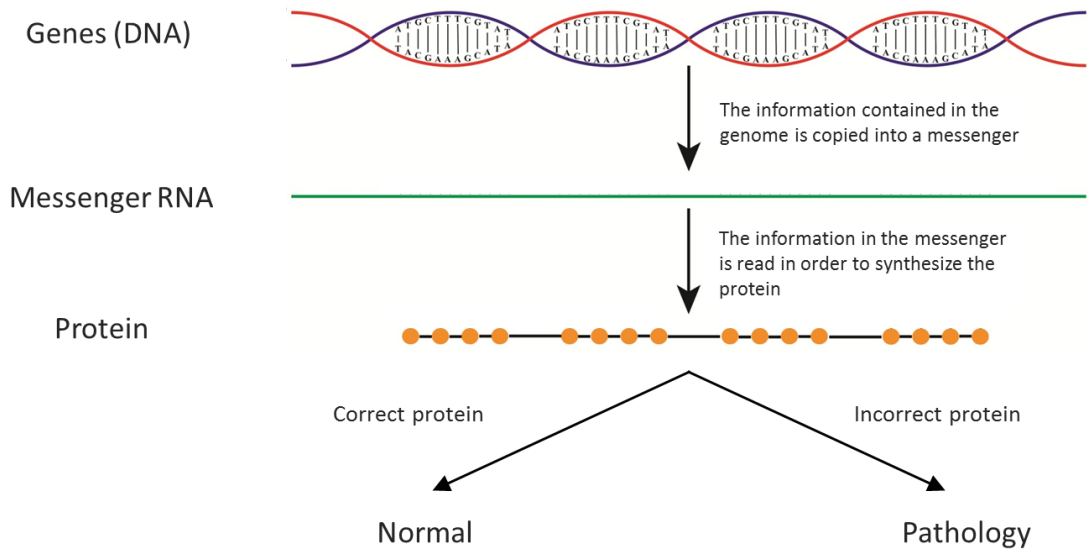
The mutations that cause the pathology are those that produce a protein that is assembly-competent (that is, functional enough to assemble with the rest of the chains), but unable to form a normal protein complex structure. In these cases, which are called dominant negative recessive mutations, the mutated protein has a toxic effect as it interferes with the normal function of the correct copy. The disease is therefore not caused by the absence of a protein, but, on the contrary, its presence in the cells hampers the function of normal proteins.

The severity of the pathology depends on the specific mutation and the degree of "defectiveness" of the collagen chain produced by the mutated gene. Think of it as a rope. If we have a loosely braided rope, it will not hold as much weight as a perfectly manufactured rope. But how much weight it can effectively support will depend on how much the rope frays. If it is just a bit loose you may not even notice the difference, but if the threads are all going in different directions, it will likely snap the moment we pull it tight.

Current treatment approaches to COL6-CMD

The protein production process is common to all the cells and to all the proteins in the organism and is key to understanding the different therapeutic alternatives.

In short, genes encoded in the DNA are copied into a messenger in order to transfer the information from the DNA to the machinery that actually manufactures the proteins just like a cooking recipe. The messenger is read and, based on the information it contains, the corresponding protein is manufactured by the cell. If there is an error in the DNA (e.g., a mutation), it will be copied into the messenger and the "recipe" for the protein will be wrong, causing the final product (the protein) not to be correct and thus causing the disease.



To address the treatment of a genetic disorder such as COL6-CMD, therefore, we can tackle any point of this process, from correcting the error at the DNA level, to compensating for the effect that the defective protein causes in the organism. The closer we get to the DNA, the more definitive the cure will be, but at the same time the harder to achieve.

1. Compensating for the effect of the defective protein

A first approach is to address the effect caused by the defective protein in the body, specifically, in muscle tissue.

Remember that one of the effects of the disease is that it increases the level of apoptosis in the muscle. Apoptosis means “programmed cell death” and consists of an organized mechanism of self-destruction that all cells have incorporated. This mechanism is useful when a cell "strays" from the normal pathway (as, for instance, a cancer cell that grows without control), at which point the auto-switch-off function kicks in. If, on the other hand, the cell is behaving normally and is needed to carry out its function, the organism only has to send the cell constant signals of survival to prevent the process of self-destruction from being triggered. These signals reach the cell through the extracellular matrix. Therefore, when the extracellular matrix (formed, among others, by collagen) does not work properly the survival signals cannot reach the cell with the appropriate intensity and it ends up self-destructing earlier than it should. That is the reason why patients with a deficit of COL6, in whom the collagen defect causes the extracellular matrix not to work properly, muscle cells see their number reduced progressively due to an increased level of self-destruction.

In stopping or reducing apoptosis in the muscle, so far, the most promising drug is omigapil. Omigapil is a product developed years ago by the pharmaceutical company Novartis for Parkinson's disease and Amyotrophic Lateral Sclerosis (ALS). However, the drug did not show any positive effects in these

patients. However, some time later, the Swiss company Santhera, seeing that omigapil had shown a blocking effect on apoptosis, resumed the development of the drug, but this time for the treatment of congenital muscular dystrophies such as COL6-CMD and LAMA2-CMD (which causes a similar but more severe form of the disease).

So far, a single clinical trial with omigapil has been conducted at the *National Institutes of Health* (NIH) in the United States, including 24 patients between 6 and 16 years (12 with COL6-CMD and 12 with LAMA2-CMD). The result of this trial is expected to be published by the end of summer 2018 but Dr. Carsten Bönnemann gave us heads up saying that the product has shown to safe for administration in children with these pathologies (no relevant adverse effects were reported during the 3-month study period). In addition, the study allowed researchers to determine the most appropriate dose that should be used in subsequent trials that are necessary to get drug approved for clinical use.

The next step in the Omigapil Project, therefore, is to conduct a new clinical trial that proves the drug effectively provides a clinical improvement in patients (an efficacy study). This, which shall constitute the phase II of the drug development process, will be carried out in the near future (the protocol is awaiting review by an expert committee that is scheduled to meet in the fall of 2018), and it will include a number of different clinical centers at international level, from the US to Europe. All going well, the results could see the light by the end of 2019, since the effect is not expected to be immediate and, thus, the treatment period will need to span between 6 months and 1 year.

The effectiveness we can expect from this drug is still to be seen, though. If, in fact, it has positive effects on the patients, we could be looking at a drug that, on the least, slows down or even stops the process of degeneration of the muscle tissue. In an optimal scenario, it might be able to reverse some of the muscle deterioration as some preclinical data suggest that omigapil reduces the fibrosis that, in time, occurs in the extracellular matrix as part of the degenerative process.

Nevertheless, the long-term effect of the drug it is still unknown. Since apoptosis is a sort of defense mechanism for the organism, shutting it down may cause undesired effects. In this regard, researchers argue that apoptosis is triggered through multiple pathways and, thus, is unlikely to be shut off entirely. This means that, while the drug cannot be 100% effective because it does not cancel all apoptosis signals in muscle cells (which is the idea behind the therapy), it does allow the process to occur when necessary in other tissues (which is desirable to avoid unwanted adverse effects).

Although omigapil it is a promising therapy, we must keep in mind that it does not address the root of the problem, but only intends to palliate the effects of the defective protein the body keeps producing. It is, however, a chronic treatment that may help buy time while new, more definitive therapeutic approaches are developed, and, in any case, shall complement other therapies if it can improve the resistance of muscle tissue to degradation. Also, it is very likely that new drugs will soon appear which, without being specific to muscular dystrophies, address common symptoms and may also be used as part of the available therapeutic arsenal for these patients.

2. One step up: blocking the production of the defective version of COL6

One of the alternatives to correct a genetic defect usually consists in introducing a correct copy of the mutated gene into the body. Unfortunately, in COL6-CMD, because the mutated copy of the gene has a toxic effect on the correct copies, this approach is useless since it does not prevent the production of the defective protein.

However, patients abnormally lacking one copy of the gene do not show symptoms of the pathology. Therefore, since we all have two copies of each gene, if the expression of the defective copy could be blocked, even if only one of the two copies remained expressed, the pathology could be effectively stopped.

With this in mind, several techniques are being developed to specifically inhibit the reading of the messenger containing the error, while allowing the correct copy to be read normally.

This can be achieved by administering small RNA fragments (oligonucleotides or antisense RNA, similar to the messenger but much shorter) that bind to the messenger and block the reading process,

thus preventing the production of the defective protein. These fragments have a highly specific sequence so that they only recognize the mutated messenger and ignore the normal copy.

The technique involves taking a short DNA sequence specifically designed against the mutated copy of the COL6 gene. This genomic sequence is then encapsulated into a virus, which, after being injected into the subject, will transport the new genetic material to the muscle cell and release it. This new genetic material will be read just like the normal cell genes and will produce the sequence (called siRNA) that will bind to the defective messenger and block its expression. The virus used for this approach (known as Adeno-Associated virus or AAV) is programmed specifically to target muscle cells and not to infect other types of cells, so the therapy affects exclusively the target tissue (the muscle) avoiding undesired effects in other parts of the body.

The negative part of this approach is that the siRNA that is introduced into the virus must be designed specifically for each individual patient, since each subject may have a different mutation (while there are some mutations that are much more frequent than others, there is still a degree of variability). This makes creating a therapy that can generically serve all COL6-CMD patients very difficult. However, scientists are already working to find ways to make this technique applicable as universally as possible so the most patients can benefit from the same therapy.

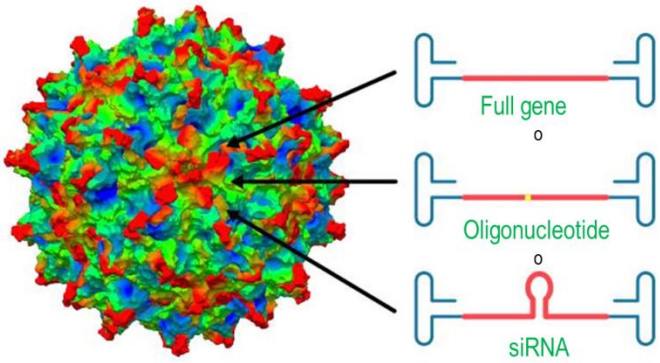


Fig. 2

AAV virus used as a vector to transport the new genetic material (be it an entire gene, a fragment of it or an oligonucleotide) to the target cells

The new genetic material carried by the AAV virus is not inserted into the cell DNA, but is only delivered to the cell nucleus where it sits along the cell's original genes. However, one limitation of the current design lies in the fact that the new DNA is not replicated when the cell divides and, thus, is not transmitted to new cells. Consequently, the effect slowly wears off and subsequent doses are required.

This approach, nevertheless, entails a problem that researchers have already taken into consideration although not fully solved. Because the therapeutic genetic material is administered inside a virus, the immune system may attack it the same way it fights any virus (flu, cold, etc.) that enters the body. Given that the AAV virus used is relatively common (albeit harmless), it is estimated that between 20% and 30% of school-age children may have been exposed to it, so their immune system will destroy the virus and its content before it can reach its destination and the therapy will have no effect. By the same token, after the first dose the patient's immune system will be primed and ready to recognize and destroy the foreign element (the AAV virus) and, again, subsequent administrations of the therapy will be rendered useless.

To overcome this obstacle, so far, two options have arisen. The first one consists in using a different strain of the virus (the same virus but with small differences that prevent the immune system from recognizing it) to administer each subsequent dose. In this way, the modified virus would travel to the target cells unnoticed by the immune system. The other option is to administer immunosuppressant drugs (as in transplanted patients), which temporarily block certain functions of the immune system, in order to give the virus time to reach the muscle and deliver its payload, and then withdraw the drug to avoid impairing the patient's defenses for too long.

Currently, this technique based on introducing foreign DNA to block the production of the defective protein is being tested in patients of Duchenne muscular dystrophy and Amyotrophic Lateral Sclerosis (both much more serious diseases than COL6-CMD). They are receiving the therapy anywhere from 1 time per month to every 4 months, depending on the case. However, Dr. Bönnemann points out that this approach is not fully optimized and there is still room for much improvement, from the design of the oligonucleotide sequences to the synthesis process, both of which can greatly improve the outcome of the therapy.

For practical purposes and as far as we are concerned, so far, in the area of COL6-CMD, this therapeutic approach has only been tested *in vitro* (that is, in the laboratory, in a test tube, using mouse cells in which the mutation had been previously introduced). In these tests, over 90% of the production of the defective protein was blocked, which is tremendously promising.

Additionally, as a next step, in Dr. Bönnemann's laboratory, genetically modified mice with known mutations in the COL6 gene have been obtained, with the aim of testing this particular therapy in an environment closer to the human body.

When asked about the time it will take these trials to reach the clinical phase, Dr. Bönnemann pointed to a time frame of about 3 years, during which he also foresees the oligonucleotide production process improving enough to offer much better chances of success in human trials.

But for a therapy to be used regularly outside of clinical trials, it needs to be approved by health authorities. Since each patient has a mutation that may be different, one might think that each patient treatment could be considered as a new drug and, therefore, subject to the vast number of tests that are required to be approved for clinical use.

As this regards, Dr. Bönnemann said that the regulatory approach currently under discussion with the US Food and Drug Administration (FDA) for these therapies is that, once it has been demonstrated that the technique works and is safe for patients, a class approval could be obtained, that is, an approval of the procedure rather than of the product itself. This would authorize the use of the therapy regardless of the specific DNA sequence that is loaded into the virus.

3. The definitive solution: correcting the mutation

The ultimate frontier is, of course, to get to the root of the problem and reverse the genetic defect of the DNA, either by correcting the wrong sequence or, as we have seen previously, entirely deleting the faulty copy of the COL6 gene so that only the correct copy remains to be expressed.

From the technical point of view, there are tools that, in theory, could do that job. The most actively explored in genetics, either as a laboratory tool or as therapeutic platform, is CRISPR/Cas9.

Without going into details that fall outside the scope of this article, we could say that CRISPR/Cas9 is a sort of biochemical scissors that can cut DNA. Properly designed, CRISPR/Cas9 cuts and re-joins pre-defined sections of the genome to delete parts of the sequence or even a whole gene.

However, to this day this approach has significant limitations. On the one hand, its efficiency is very low, reaching only 2-3% of the treated cells (remember that the oligonucleotides therapy blocked over 90% of the faulty gene expression). Furthermore, we must keep in mind that modifying the DNA is an irreversible process that has to be executed very carefully. Any mistake can be fatal so, beyond making sure that the DNA is cut exactly where we need to, we must guarantee that the tool does not cut anywhere else, however similar the sequence may be.

In short, this approach is ambitious in terms of the result it pursues, but it is still too early to be seriously considered as a therapeutic option.

Can food and supplements help?

A number of nutritional supplement and/or dietary approaches have been suggested to contribute to an improvement of the patients' condition. However, so far, no clear evidence has been presented that can support the standard use of any of those alternatives.

Spermidine

This compound is a biochemical compound that is known to be responsible for a wide variety of processes within the organism, among others, the regulation of autophagy. Autophagy is a self-degradation process responsible for the clearance of damaged or unnecessary cellular components (in layman's terms, a natural waste removal process at the cell level). It has been shown that an excess of cellular waste in the form of dysfunctional organelles due to autophagy failure is a key event in the pathogenesis of COL6-CMD. Some data suggest that spermidine has a positive effect on muscular dystrophy by increasing autophagy. Therefore, in theory, it could alleviate the unwanted biochemical consequences of the disease. However, while there is suggestive evidence, there is not yet enough data to endorse its regular use as a therapy co-adjuvant in COL6-CMD patients.

Low-protein diet

In terms of potential dietary regimes that may improve patients' condition the same way that certain foods are believed to help in cancer treatment, it has been suggested that a low-protein diet can, in a similar way to spermidine, positively affect the autophagy process, thus fighting some of the biochemical disorder caused by the pathology. Again there is, regretfully, not enough evidence to conclude that it is an effective approach and, since CMD patients are particularly low on muscular mass, depriving them of the raw material for muscle synthesis (dietary protein) does not seem like a good idea at this point.

Collagen supplements

While it may seem sensible to supplement the diet of a collagen-deprived individual through dietary supplementation, we need to understand that endogenous collagen 8the collagen synthesized by the organism where it is needed) is nothing like the collagen we may ingest as a supplement. On the one hand the digestive process will cut and destroy the ingested collagen just as it does with any other protein we might eat. Secondly, as we have seen, there a many types of collagen and we are interested in only one of such types, collagen VI, which is unlikely to be present in significant amounts in the preparation, if at all. Finally, even if we get past these two hurdles, there is the question of whether the collagen intake will actually reach the muscle, where we need it, and in which amounts, rather than be distributes throughout the organism. All in all, the idea of a collagen dietary supplement being an effective contribution to patients' wellbeing is quite far-fetched.

Conclusion

We have seen that there are indeed several approaches to the treatment of COL6-CMD, a disease that, so far has no identified cure. This is due to its genetic origin which, as in many other medical conditions, represents a major challenge for medical therapy.

However, research is advancing at enormous speed in this field and every day new initiatives and therapeutic options arise. Although, regretfully, they do not progress as fast as families would like, there is no denying that the speed and results are more than remarkable. Each achievement is undoubtedly a new weapon in the fight against these rare diseases and a new tool to buy time until the ultimate solution is found, both for COL6-CMD as well as many other congenital diseases.