

Press release
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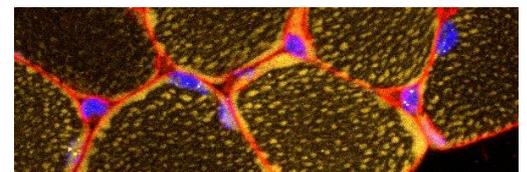
CRISPR-Cas9 First *in vivo* proof-of-concept in Steinert's myotonic dystrophy, a neuromuscular disease

Ana Buj Bello's team, a researcher in an Inserm unit at Genethon, the AFM-Telethon laboratory, has made the proof-of-concept of a CRISPR-Cas9 approach in a mouse model of Steinert's myotonic dystrophy, the most common neuromuscular disease in adults. Indeed, thanks to this genome editing approach, the expanded CTG triplet repeat in the *DMPK* gene, which is responsible for the disease, was "cut" and removed from the gene, and the number of toxic RNA aggregates was decreased in the muscle cells of the tested models. Based on these encouraging results, published in the June 5 issue of *Molecular Therapy*, the researchers are currently investigating whole body treatment.

Steinert's myotonic dystrophy (DM1) is the most common form of adult muscular dystrophies. With a genetic origin, its prevalence is estimated at 1 in 8,000 persons, and is mainly characterized by difficulties in relaxing after contraction (myotonia) and a progressive muscle weakness. The disease is due to mutations in the *DMPK* gene, in particular, to an increase in the number of repeats of a small 3-nucleotide DNA sequence, a CTG triplet, located in the *DMPK* gene. This results in the accumulation of mutated *DMPK* RNA in the cell nucleus, leading to alterations in cellular functions. There is currently no cure for this neuromuscular disease.

In this study, Ana Buj Bello's team, in collaboration with Denis Furling's team at the Institute of Myology and Genevieve Gourdon's team at the Imagine Institute, have developed and evaluated a gene therapy approach using the CRISPR-Cas9 molecular scissors in cellular and *in vivo* mouse (DMSXL) models of the disease:

→ **In the cellular model** : The team has identified guide RNAs that target DNA sequences around the CTG triplet region of the *DMPK* gene, and demonstrated that the Cas9 protein removes this part of the genome, resulting in the disappearance of nuclear toxic RNA aggregates in treated cells.



Nucleus (blue) with mutated DMPK RNA aggregates in muscle fibers

→ **In the mouse model** : The team has used the same technology *in vivo* and administrated intramuscularly gene therapy vectors (AAV9) that carry Cas9 and guide RNAs targeting the *DMPK* gene. Few weeks after injection, a decrease in toxic RNA aggregates was observed in the nuclei of muscle cells of diseased mice.



"After several years of work, we have been able to demonstrate that the CRISPR-Cas9 system works in skeletal muscle for Steinert's disease. These results represent a very encouraging first step, and we now plan to optimize this approach to correct the entire musculature and other tissues affected by the disease", highlights Ana Buj-Bello, lead author of the work.

Publication : Genome editing of expanded CTG repeats within the human DMPK gene reduces nuclear RNA foci in muscle of DM1 mice - Mirella Lo Scudato¹, Karine Poulard¹, Célia Sourd¹, Stéphanie Tomé², Arnaud F. Klein³, Guillaume Corre¹, Aline Huguet², Denis Furling³, Geneviève Gourdon², and Ana Buj-Bello¹

Affiliations : ¹ Genethon, UMR_S951 Inserm, Univ Evry, Université Paris Saclay, F-91000 Evry, France; ² INSERM UMR 1163, Institut Imagine, Université Paris Descartes-Sorbonne Paris Cité, F-75015 Paris, France; ³ Sorbonne Université, INSERM, Association Institut de Myologie, Centre de Recherche en Myologie, F-75013 Paris, France

About Genethon – www.genethon.fr

Created and financed by AFM-Téléthon, Genethon aims to provide patients with innovative gene therapy treatments. Having played a pioneering role in deciphering the human genome, Genethon now employs close to 180 researchers, doctors, engineers and regulatory affairs specialists, and is one of the leading international centres for preclinical and clinical research and development in gene therapy treatments for rare diseases. 8 products resulting from Genethon's research are currently being tested in the clinical trials.

About AFM-Telethon – www.afm-telethon.fr

AFM-Telethon is an association of patients and their relatives, committed to fighting disease. Thanks to donations from the Téléthon (€85.8 million in 2018), it has become a major player in biomedical research into rare diseases in France and across the world. Today, it supports clinical trials testing treatments for genetic diseases of the eyes, blood, brain, immune system and muscles. It is unlike other associations in that its laboratories have the ability to design, produce and test their own innovative therapies. Free telephone number for affected families: 0800 35 36 37

Press contacts:

Stéphanie Bardon, Marion Delbouis
+(33)01.69.47.29.01 – 06.45.15.95.87 – presse@afm-telethon.fr