RESEARCH UPDATE: DEVELOPMENT OF THE FIRST HUMAN CELLULAR MODEL TO STUDY CYSTINOSIS MYOPATHY

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The need for research on cystinosis muscle weakness

Cystinosis is an **ultra-rare genetic disease** which mainly affects the kidneys and eyes. Consequently, most current research on the disease focuses on these clinical features. But cystinosis affects all body cells, and people living with the disease can also develop muscle weakness (**myopathy**). The underlying mechanisms for the development of myopathy are not understood. Studies showed that the only current treatment for cystinosis, **cysteamine**, does not prevent muscular dysfunction, which manifests around the second decade of life. Cystinosis is associated with decreased muscle strength due to both low muscle mass and poor muscle quality, which can negatively affect the quality of life of people living with cystinosis in performing daily activities independently, with varying severity. The prevalence of cystinosis muscle weakness varies across studies (20-75%), likely resulting from differences in patient demographics, assessment scores and cysteamine regimens among older populations. Four adults living with cystinosis decided to share how myopathy affects their everyday lives:

- Ernesto, 35: Cystinosis-related myopathy means having increased dependency on help for daily activities, such as opening bottles (including medication) and doing household chores. It takes longer to dress and undress myself, and lately I've been finding personal hygiene tasks increasingly difficult. Also, during the winter, muscle mass loss makes my hands feel frozen and occasionally painful.

- Marta, 21: My muscular issue is mild oropharyngeal dysphagia, which causes insufficient strength to swallow solid food, and occasional choking or even vomiting. I always need to have water at hand when eating, and sometimes I need to drink so much that I get full quickly, and then I am not hungry anymore. I also need to eat very carefully and slowly, because I choke easily. This is also an issue in everyday life because at some jobs, you have little time available for lunch.

- Freek, 46: Myopathy is one of the factors that has affected my lung function, which is currently around half. Muscle strength is something you must train all the time. But also, you cannot really do anything about it, especially in the mouth and lungs.

- Alba, 37: As a child, I only noticed hyperflexibility in my hands, but over time, I developed difficulty with finger movements and muscle wasting. I try to compensate for my low muscle mass, though it remains challenging, and I wish I had earlier information to understand what was happening and why.

Despite prevention and treatment of myopathy being an unmet need in cystinosis, there is a lack of fundamental research to study how cystinosis affects the muscles. Consequently, a collaborative research project mainly developed at the KU Leuven (Belgium) with the research support of Cystinosis Ireland and Cystinosis Foundation UK, invested in developing the first human muscle cell model for cystinosis: "*An Isogenic Human Myoblast Cell Model for Cystinosis Myopathy Reveals Alteration of Key Myogenic Regulatory Proteins*" (https://doi.org/10.1002/jcsm.70116).

The results from the work

The main limitation of **cysteamine** treatment is that it does not address the underlying genetic cause of the disease. Cystinosis is caused by mutations in the ***CTNS*** gene that provides instructions for making a protein whose job is to remove **cystine** (a building block of proteins) from tiny storage compartments inside our cells called **lysosomes**. In people with cystinosis, the faulty ***CTNS*** gene leads to cystine accumulation, which in turn impairs cell functions, resulting in loss of kidney function and decreased muscle strength, for example.

In this work, Louise Medaer and Roger Mora, both doctoral candidates in the group of Prof. Gijsbers and Sampaolesi at the KU Leuven, used human immature muscle cells and grew them in the lab (***in vitro***) to mimic the first steps of the process in which our bodies make muscles (**myogenesis**). **Myogenesis** is a lifelong process that allows skeletal muscle to regenerate and adapt after damage, such as the small injuries caused by exercise or intense muscle use.

Using gene editing strategies (CRISPR Cas), they inactivated the functional ***CTNS*** gene, causing the muscle cells to accumulate cystine, the hallmark of cystinosis. In addition, they observed that immature muscle cells (myoblasts) lacking a functional ***CTNS*** gene had slightly lower efficacy to merge after maturing into early muscle fibres (myotubes). Moreover, specific cell machinery key for muscle cell formation was underrepresented in cystinosis myotubes compared to unaffected myotubes.

To underscore that the observed effect is solely caused by the loss of a functional ***CTNS***, they used a **gene addition approach** to add the coding sequence of ***CTNS*** back to cystinosis cells. Importantly, this reversed all the observed effects in cystinosis muscle cells. Finally, they used different techniques to get a full picture of how cells work (**multi-omics**). This corroborated that the lack of a functional ***CTNS*** gene mildly lowered cell machinery responsible for muscle cell development. These differences were reverted upon reintroduction of a functional copy of the ***CTNS*** coding information.

Discussion

This study suggests that, in an ***in vitro*** model, the loss of a functional ***CTNS*** can mildly impair the first steps of muscle development (**myogenesis**). It is important to acknowledge the limitations of cell culture models, which cannot fully replicate the complexity of muscle biology in the human body. The relevance of this work is the creation of the first human cellular model to study **cystinosis myopathy**. This model can be the first step towards developing better platforms to both understand the disease mechanism, underlying pathways and to test novel therapies, such as **gene therapies** or new small drugs.

Next steps

Building on this work, the same scientific team is currently developing a more advanced ***in vitro*** model that can better mimic muscle formation. Thus, they aim to better understand how cystinosis affects muscle cells and what the impact of cysteamine treatment is. In addition, they are teaming up with groups providing complementary skills from other universities in France and Ireland to study cystinosis myopathy in 3D mini-muscles grown in the lab, and in animal models of the disease. Cellular and **gene therapies** are relatively novel approaches, and their potential lies in targeting the underlying causes of genetic diseases with the potential to provide a cure. These ***in vitro*** models can be used to take first steps to assess **gene-based therapies**: safe modified virus-based vectors, gene editing strategies (CRISPR/Cas) and lipid-nanoparticles (LNPs) similar to COVID-19 vaccines.