**Employing isogenic cell models to study the underlying mechanism of cystinosis myopathy**

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Lay-oriented abstract:

Cystinosis is a rare, metabolomic disease, caused by lack or dysfunction of cystinosin (CTNS). This disorder is systemic, but primarily affects the kidneys resulting in severe kidney injury. Kidney transplantation, cysteamine treatment, and improved medical care have led to a better quality of life and life-expectancy for cystinosis patients. However, the latter results in the emergence of extra-renal complications in other organs, such as the muscle. Muscle weakness is primarily observed in the hands and feet and over the years can also affect swallowing and respiratory muscles. The underlying etiology is unknown.

Currently, only a very few clinical studies examined muscle weakness in cystinosis patients. No human cell models have been employed to investigate how cystinosis patients develop muscle weakness. To achieve this, we will generate human muscle cell models in which the causative *CTNS* gene is disrupted by CRISPR/Cas to assess the muscle phenotype at molecular level. The results of this project will provide us with better insights into how lack of CTNS leads to muscle weakness with the possibility to develop new therapeutics.

Scientific abstract:

Cystinosis is an autosomal recessive lysosomal storage disease caused by lack or dysfunction of cystinosin (CTNS), leading to cystine accumulation and when left untreated it will first affect the kidneys. Renal transplantation, cysteamine treatment and improved medical care led to a better quality of life and life-expectancy for cystinosis patients. However, the latter also led to the emergence of additional systemic phenotypes like myopathy. Muscle weakness forms a major concern leading to life-threatening events like swallowing difficulties and respiratory insufficiency. These complications affect over 80% of the untreated patients by the age of 40 and still affect cysteamine-treated patients although the rate of this complication is decreased. The etiology of cystinosis myopathy remains to be elucidated. This project aims to shed light on the role of CTNS by employing human muscle cell models to better understand the pathophysiology of cystinosis myopathy with the potential to develop new therapies. Patient-derived immortalized myoblasts together with nanoblade and viral vector technology are used to install isogenic muscle models and to assess the potential of a gene addition approach, respectively. In a first step, CRISPR technology was employed to generate a polyclonal isogenic human CTNS knock-out (KO) myoblast cell model, corroborated by elevated cystine levels in metabolomic analysis, the robust hallmark of cystinosis. *CTNS* cDNA addition using lentiviral vectors reverted the cystine accumulation seen in the CTNS KO model. To further evaluate the cystinosis muscle phenotype, differentiation analysis of the myoblasts was assessed for CTNS KO, showing no significant difference in fusion index compared to the control WT myoblasts. Analysis of autophagy parameters (LC3 and P62), known to be altered in kidney cells in cystinosis, also remained unaffected.

As a next step, we are establishing 3D myoblast spheroid models to better mimic the *in vivo* pathophysiology of cystinosis myopathy.