Second 6-month Progress Report

Research Grant: Cystinosis-associated myopathy:
Impact of musclin gene therapy and exercise

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BACKGROUND AND RATIONALE

The association between cystinosis and muscle wasting and weakness represents an important clinical research focus and innovative effective and preventive therapies to improve myopathy in people with cystinosis are of utmost importance. In a previous CRF funded project, we identified for the first time that the skeletal muscle-derived myokine musclin is impaired in cystinosis. We could show that musclin synthesis is significantly reduced in wasting muscles from *Ctns*$^{-/-}$ mice, which was associated with the induction of cachectic genes suggesting a causative role of musclin deficiency in the pathogenesis of cystinosis-associated myopathy.

SPECIFIC AIMS AND CURRENT STATE OF THE STUDY

The central hypothesis of this translational new research project is that re-induction of musclin restores muscle strength and overall muscle health and thereby improves myopathy in cystinosis. Here we propose to investigate in Specific Aim #1 if an **AAV-based gene therapeutic re-expression of musclin regenerates muscle strength and improves overall muscle health in *Ctns*$^{-/-}$ mice** and in Specific Aim #2 whether **re-induction of musclin by exercise also has a positive effect and ameliorates cystinosis associated myopathy (Figure 1)**.

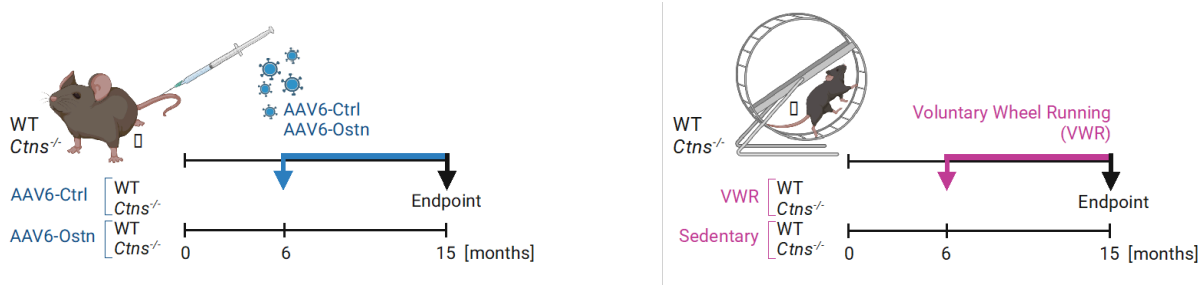

Figure 1. Study designs for Specific Aims #1 and #2.

Current state of the study

The breeding of the mice to generate the appropriate number of experimental animals is ongoing and working very well. Currently, we have achieved a total of n=97 mice (n=64 *Ctns*$^{-/-}$; n=33 WT), n=32 with the age required to get started on the therapeutic intervention. N=25 mice will enter the experiment in the first week of June and another n=40 over the course of the following weeks. For Specific Aim #1, each 0.5E+11 vg AAV6-Ctrl and AAV6-Ostn have been injected into the

corresponding animal groups, respectively (see Figure 1, left). For Specific Aim #2, two animal groups have started sports therapy and therefore sit in cages with running wheels (see Figure 1, right). In parallel, breeding continues until all experimental groups are filled.
The treadmill system from BIOSEB, kindly funded by the CRF, has been successfully established and initial baseline data at the start of therapeutic interventions has been generated. In addition, the baseline measurements of body weight, kidney function demomstrated by GFR measurement, grip strength and treadmill running distance were carried out. As expected, at the start of therapeutic intervention, WT and *Ctns*$^{-/-}$ showed an equal body weight, GFR and grip strength (Figure 2). Only the running distance in meters was slightly reduced in the *Ctns*$^{-/-}$ group, but this was not statistically significant.

Figure 2. Baseline data at the start of therapeutic intervention for body weight, GFR, grip strength and treadmill running distance of WT and *Ctns*$^{-/-}$ mice for Specific Aims #1 and #2.

In summary, the baseline data has been collected and therapeutic intervention for Specific Aims #1 and #2 has begun. The different experimental groups will continue to receive each respective treatment for a period of 9 months in total, which is expected to end at the turn of the year. In the meantime, we will conduct examinations für Specific Aim #3 regarding the investigation whether musclin enhances mitochondrial biogenesis in skeletal muscles of *Ctns*$^{-/-}$ mice thereby contributing to an improvement of cystinosis-associated myopathy.

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