

## Cystinosis Research Foundation Progress Report

### ***Energy homeostasis and muscle wasting in nephropathic cystinosis***

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### **Final Report**

#### **Overview**

Cystinosis is an autosomal recessive disorder, caused by mutations of the lysosomal cystine carrier protein, cystinosin, encoded by the CTNS gene. The intralysosomal cystine accumulation in cystinosis leads to chronic kidney disease (CKD) and multi-organ damage. Growth retardation and generalized muscle wasting are among the commonest clinical characteristics of the disorder. These complications have profound adverse effects on the quality of life. Children with cystinosis often fail to thrive before the onset of advanced CKD, suggesting that factors other than CKD might contribute to the early onset of growth retardation in cystinosis. The underlying mechanism of this growth retardation is not well understood.

#### **Hypothesis**

We hypothesized that abnormal energy homeostasis is present early in the natural history of cystinosis, before the onset of CKD, and may contribute to the profound growth retardation in nephropathic cystinosis. Inflammation is an important pathogenetic factor in the abnormal energy homeostasis and muscle wasting as well as CKD progression in nephropathic cystinosis.

#### **Strategy**

Three specific aims have been proposed to test our hypothesis.

- 1) To characterize the onset and time course of abnormal energy homeostasis, growth retardation and muscle wasting in the Ctns knockout mice, which is an established animal model of cystinosis, in the first year of life. This will be compared with wild type mice as well as the Ctns<sup>-/-</sup>-IL-6<sup>-/-</sup> double knockout mice. We will measure food consumption, body weight and length, resting metabolic rate, and body composition. Five time points will be studied, 1 month, 2 month, 4 month, 8 month and 12 month. We will study both genders.
- 2) To characterize the onset and time course of progressive CKD in the Ctns knockout mice in the first year of life. This will be compared with wild type mice as well as the Ctns<sup>-/-</sup>-IL-6<sup>-/-</sup> double knockout mice. Serum BUN, creatinine, electrolytes including bicarbonate to assess acid-base balance, 24 hr creatinine clearance and 24 hr urine protein will be measured.
- 3) To characterize the molecular pathways underlying abnormal energy homeostasis and muscle wasting in the Ctns knockout mice in the first year of life compared with wild type mice as well as the Ctns<sup>-/-</sup>-IL-6<sup>-/-</sup> double knockout mice. Both mRNA and protein expression of key molecules which regulate energy homeostasis and muscle mass will be studied.

## Progress

### 1. *Setting up and breeding of Ctns<sup>-/-</sup> mice*

We initiated the material transferring process for the breeding mice once this project was funded. The final approval from 3 institutions (Robert Mak, UCSD; Dr Stephanie Cherqui, Scripp Research Institute and Dr Corinne Antignac, Inserm) was obtained in April 2010. We received a pair of Ctns<sup>-/-</sup> mice (provided by Dr Stephanie Cherqui) in June 2010. After several months of mating, we managed to breed one male and one female Ctns<sup>-/-</sup> mice offspring in September 2010. There were some unexpected losses as the male Ctns<sup>-/-</sup> mouse breeder ate some of the offsprings. We have now employed a few strategies to combat this problem. 1/ We have now adopted the policy to separate the male Ctns<sup>-/-</sup> mouse parent from the pregnant mother and hence offsprings. 2/ We have requested additional Ctns<sup>-/-</sup> mouse breeders from Dr Cherqui and our request has been approved in November 2011. We have since actively breed those Ctns<sup>-/-</sup> mice in order to achieve a large colony of Ctns<sup>-/-</sup> mice to support the on-going experiments as outlined in our proposal. Progress of the on-going research has been listed below.

### 2. *Setting up Ctns<sup>-/-</sup>-IL-6<sup>-/-</sup> double mice*

We set to generate Ctns<sup>-/-</sup>-IL-6<sup>-/-</sup> double knockout mice. Protocol for genotyping has been optimized. We managed to generate Ctns<sup>-/-</sup>-IL-6<sup>+/-</sup> breeders so far. We crossed one Ctns<sup>-/-</sup>-IL-6<sup>+/-</sup> male and one female Ctns<sup>-/-</sup>-IL-6<sup>-/-</sup> breeder last month.

### 3. *To characterize the onset and time course of abnormal energy homeostasis, growth retardation and muscle wasting in the Ctns knockout mice in the first year of life*

We characterized Ctns<sup>-/-</sup> mice as compared to c57BL/6J (WT) mice in the first year of life. Experimental scheme is listed in Figure 1. We have measured the serum and urine chemistry, body weight, food consumption, basal metabolic rate, change in body composition, whole body bone density, *in vivo* muscle function, femur length and maximal mechanical strength, vertical length, muscle protein and gene mRNA expression in both genders at various time points (1 month, 2 month, 4 month, 8 month and 12 month).

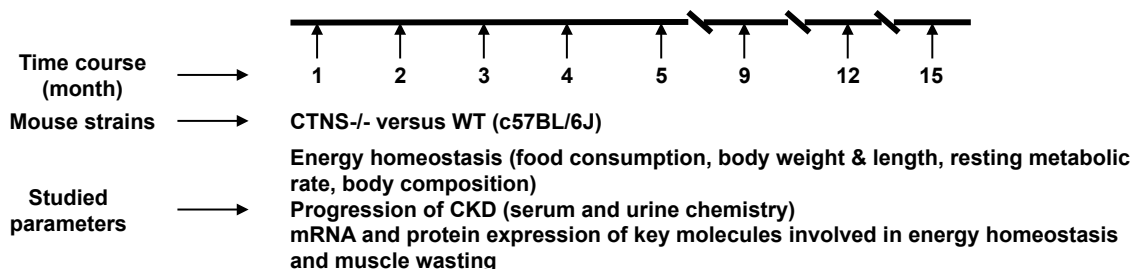


Figure 1: Experimental scheme

**Serum and urine chemistry in Ctns<sup>-/-</sup> mice:** We characterized Ctns<sup>-/-</sup> mice as compared to control C57BL/6J mice at various time points. Data are shown in Figure 2 and 3. Ctns<sup>-/-</sup> mice had higher levels of serum creatinine and BUN at the age of 4-month old and the trend persisted for the rest of the study. In addition, 24 hr urine

was also collected for further analysis. Elevated urine phosphate, urine protein and urine volume in *Ctns*<sup>-/-</sup> mice was also observed.

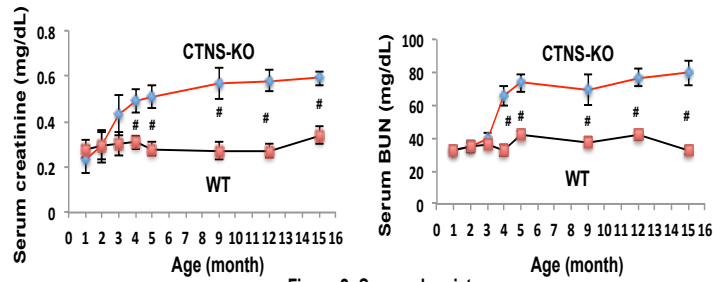


Figure 2: Serum chemistry

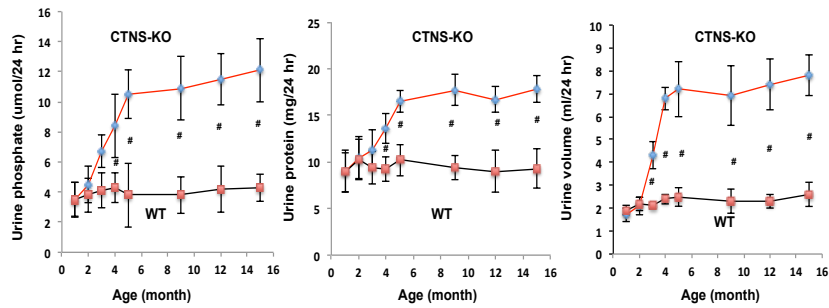


Figure 3: Urine chemistry

**Metabolic defects in *Ctns*<sup>-/-</sup> mice:** *Ctns*<sup>-/-</sup> mice gained less weight (Figure 4), consumed less food (figure 5), had higher basal metabolic rate (Figure 6) as well as lower lean mass and fat mass than age-appropriate control mice (Figure 7). *Ctns*<sup>-/-</sup> mice also exhibited a lower bone mineral density than control mice (Figure 8).

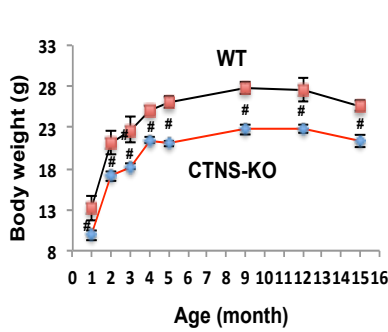


Figure 4: Body weight

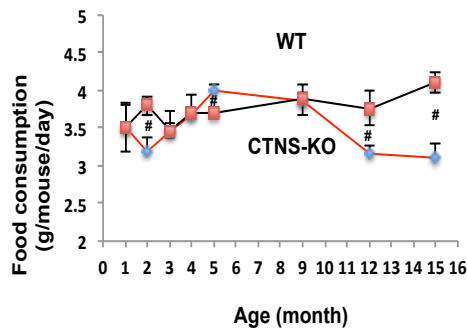


Figure 5: Daily food consumption

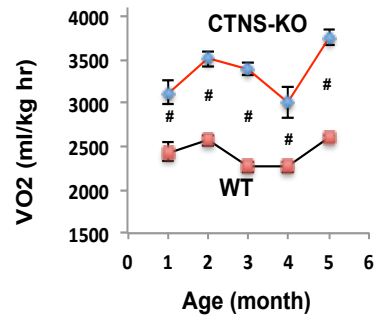


Figure 6: Basal metabolic rate

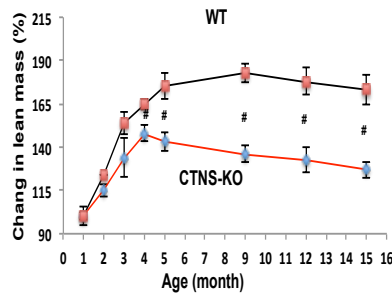
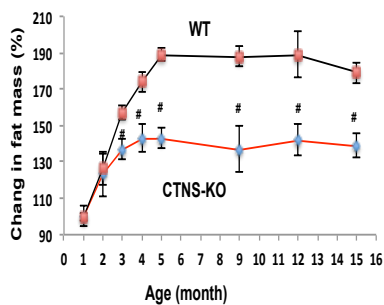


Figure 7: Body composition

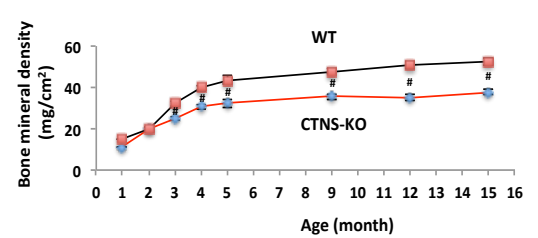


Figure 8: Bone mineral density

Muscle function, as assessed by rotarod activity and grip strength for forelimb, was significantly impaired in *Ctns*<sup>-/-</sup> mice (Figure 9). Femur length and femur maximal mechanical strength was also significantly decreased in *Ctns*<sup>-/-</sup> mice (Figure 10 and 11). Vertical length of the mouse was significantly reduced in *Ctns*<sup>-/-</sup> mice versus control mice (Figure 12).

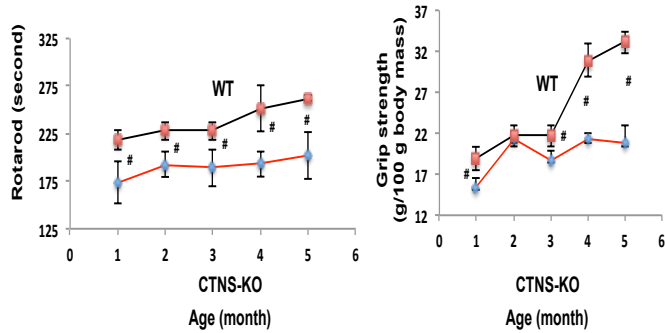


Figure 9: In vivo muscle function

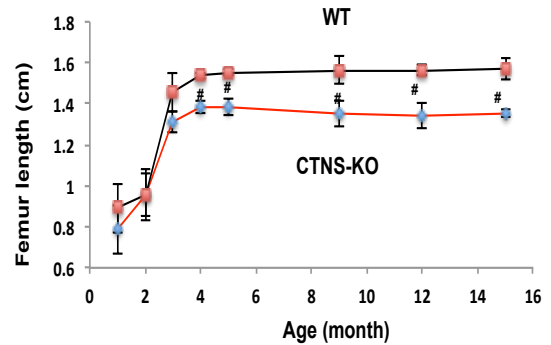


Figure 10: Femur length

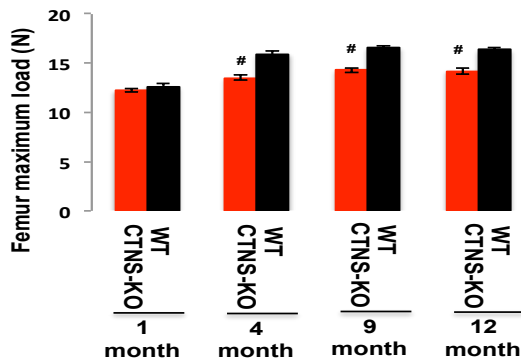


Figure 11: Femur strength

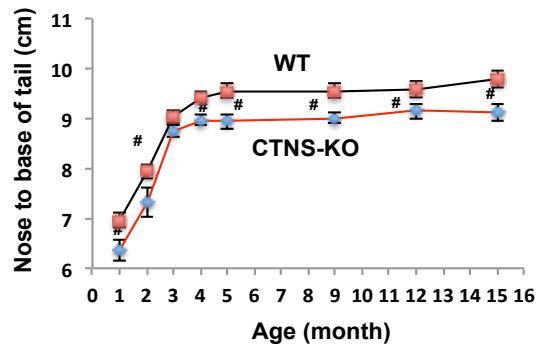


Figure 12: Vertical length

**Metabolic pathways of muscle wasting in *Ctns*<sup>-/-</sup> mice:** Protein and mRNA expression of key molecules, which regulate muscle mass are measured. Serum and muscle IL-6 levels were elevated while serum and muscle IGF-I levels were decreased in *Ctns*<sup>-/-</sup> mice from the age of 4-month versus control mice (Figure 13 and 14). Muscle Atrogin-1 and MuRF-1 mRNA levels were significantly increased in *Ctns*<sup>-/-</sup> mice than control mice. Muscle Pax-3 and MyoD mRNA levels were decreased in *Ctns*<sup>-/-</sup> mice as compared to control mice (Figure 15).

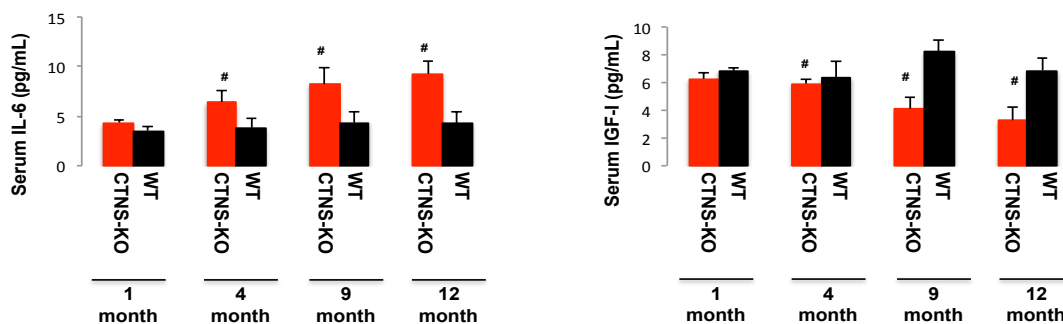


Figure 13: Serum IL-6 and IGF-I

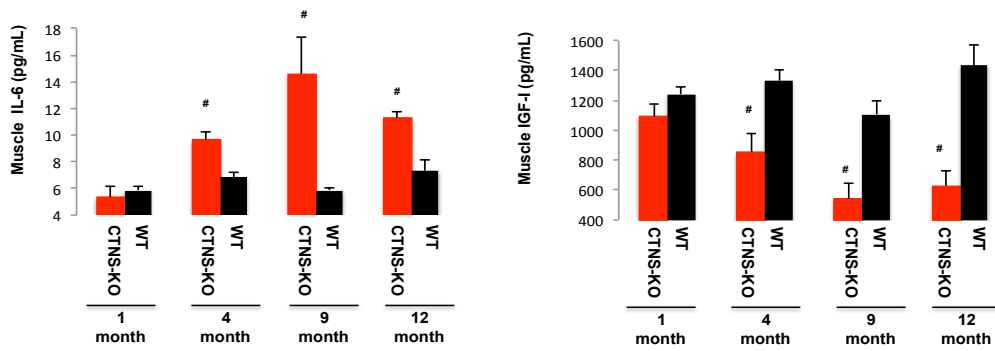


Figure 14: Muscle protein levels of IL-6 and IGF-I

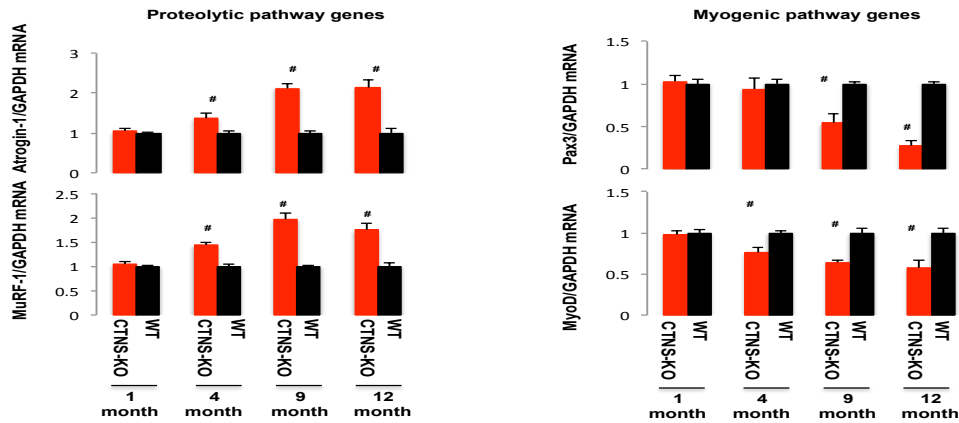


Figure 15: Muscle proteolytic and myogenic gene mRNA levels

In summary, we are able to accomplish most of the study goals, as detailed above. There are some unexpectedly delays in the breeding of the *Ctns*<sup>-/-</sup> and *Ctns*<sup>-/-</sup>*IL6*<sup>-/-</sup> mouse colony. We are in the process of preparing a manuscript for submission. We will continue to observe the onset and time course of progressive CKD in the *Ctns*<sup>-/-</sup>*IL6*<sup>-/-</sup> double knockout mice once they are available in the near future.

Sincerely,  
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