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ASSESSMENT OF MUSCLE REGENERATION IN THE R6/2 MOUSE MODEL OF HUNTINGTON'S DISEASE

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Background

**Mutant
huntingtin**



Skeletal muscle atrophy

HD pathophysiology

- Altered energy metabolism
- Type I and type II muscle fiber atrophy
- Inflammation
- Reduced muscle strength

*Van der Burg, J. M., Bjorkqvist, M. & Brundin, P, The Lancet. Neurology(2009)
Ribchester, R. R. et al. The Euro. J. of neuroscience (2004)*

Purpose of the project

Using an acute injury model, we aimed to investigate the effect of mutant HTT expression on satellite cell regeneration capacity in the

R6/2 mouse model

Methods and Materials

Cardiotoxin (CTX)

- A snake derived cytolytic toxin
- An ideal acute injury model
- Induce skeletal muscle regeneration

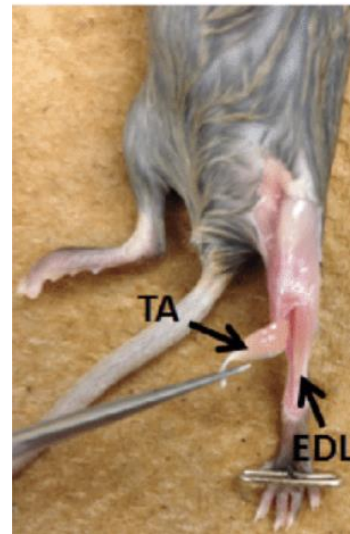
Animal groups to assess muscle pathology:

Adult: 18 weeks and neonates: 1 week

Immunohistochemistry	CTX injected animal groups
Laminin	4 weeks post injection
Embryonic myosin heavy chain (eMHC)	1 week post injection

- **Cardiotoxin (CTX) injections in Tibialis anterior (TA) muscle**

Right TA:
Saline (30µl)



Left TA: CTX (5µg)
CTX delivered in
30µl volume)

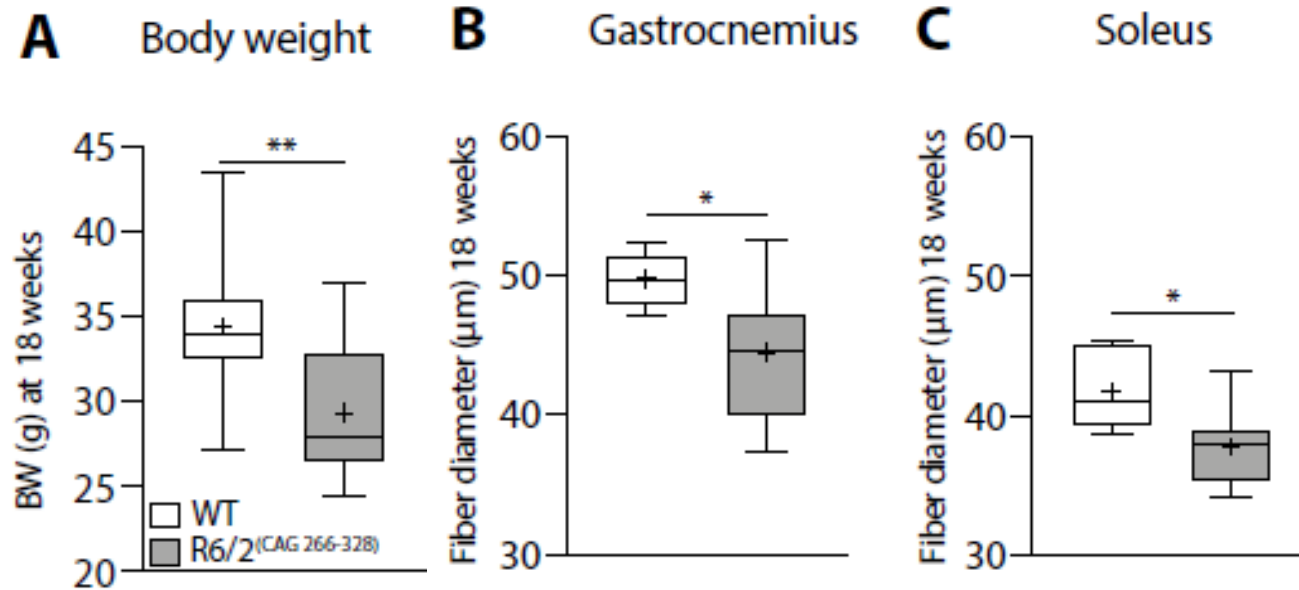
Garry, G.A. et al. Methods Mol. Biol. (2016)

Guardiola O. et al. J. Vis. Exp. (2016)

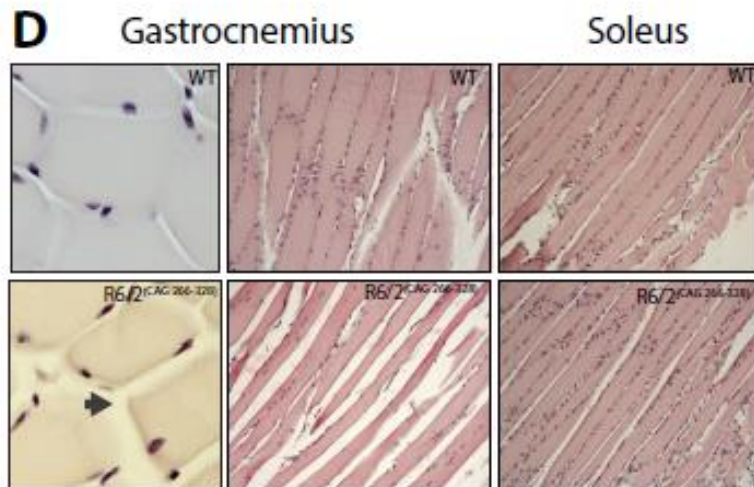
Picture taken from Shinin, Vasily et al (2009)

Results

Skeletal muscle pathology in adult R6/2 mouse model



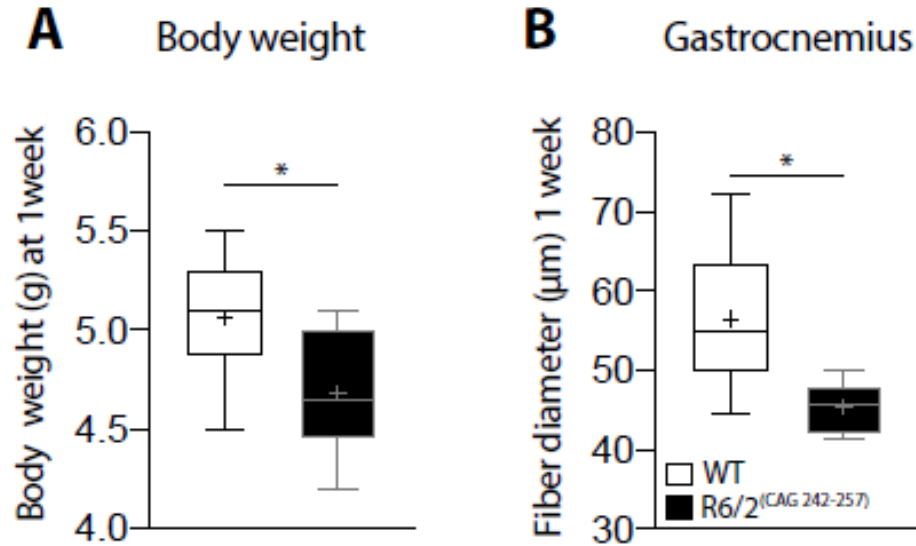
Reduced body weight and fiber diameter



Gap between fibers

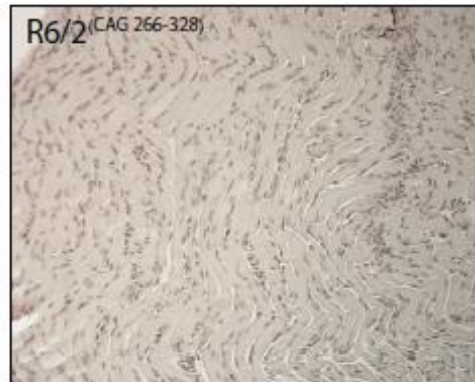
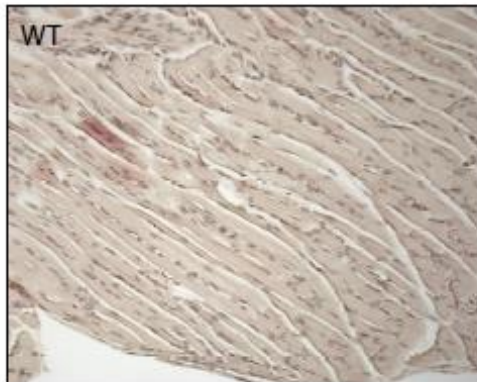
Unpublished data

Skeletal muscle pathology in neonate R6/2 mouse model



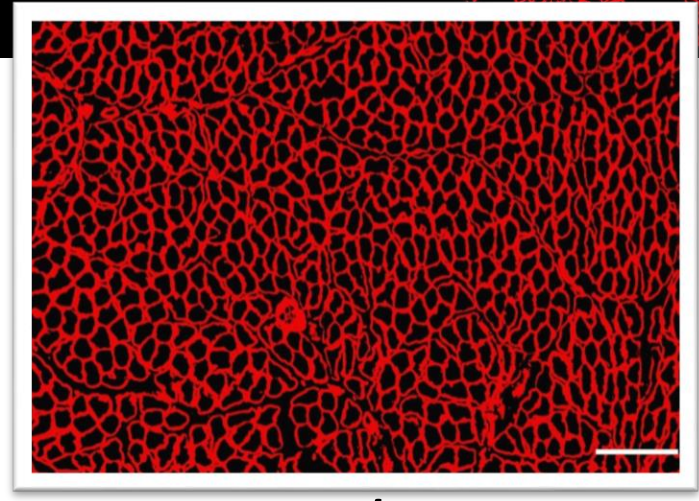
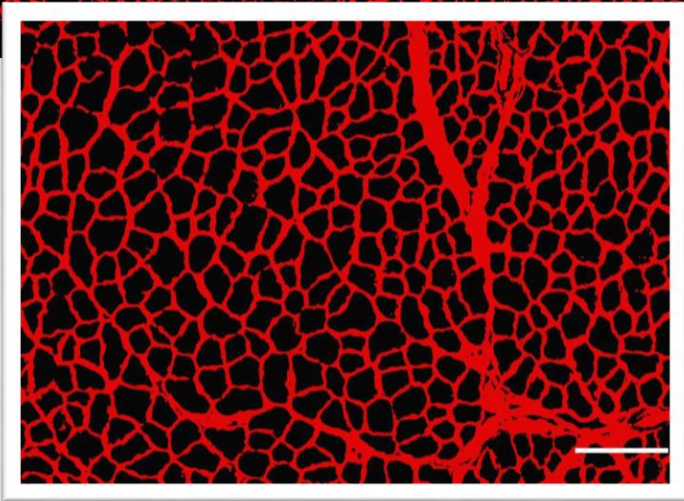
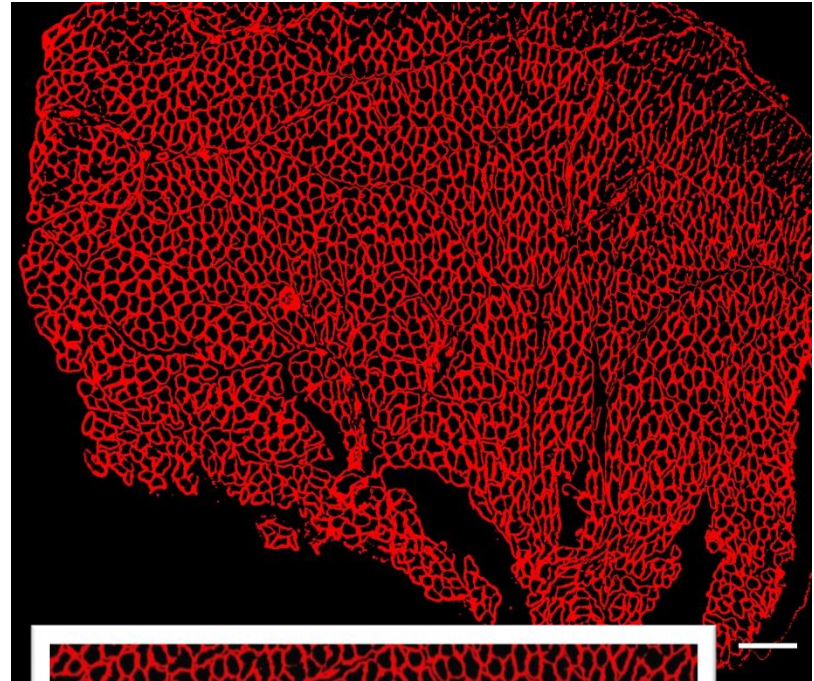
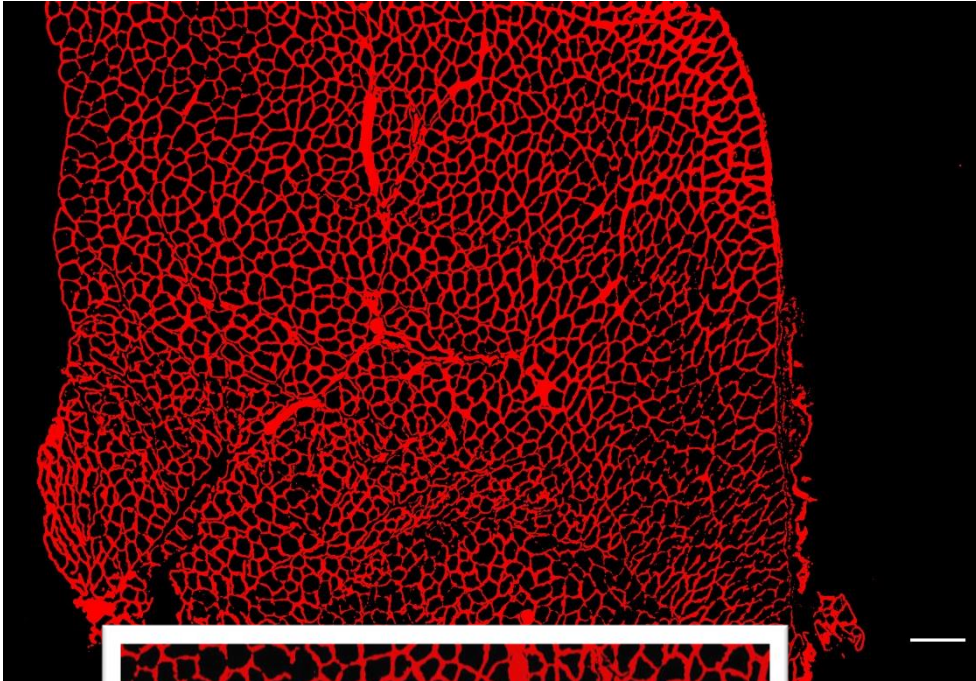
**R6/2 neonates
exhibits reduced
body weight and
fiber diameter**

C Gastrocnemius muscle (1 week old) H&E



Skeletal muscle morphology in R6/2 and WT mice

Reduced fiber diameter in R6/2 compared to WT muscle



Laminin
staining

Scale bar=100 μ m

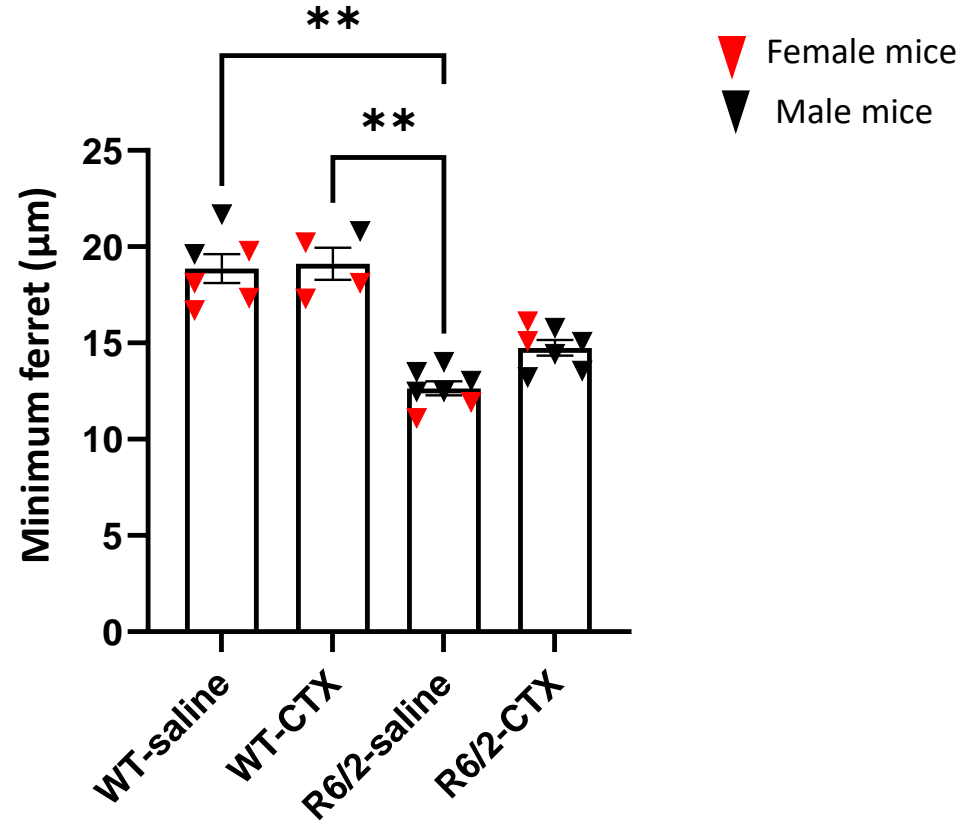
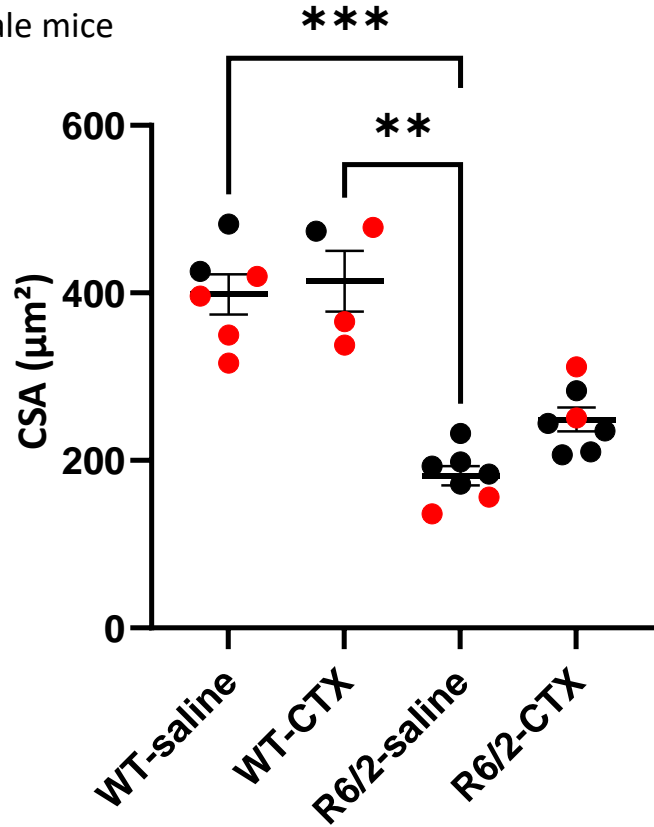
WT

R6/2

Unpublished data

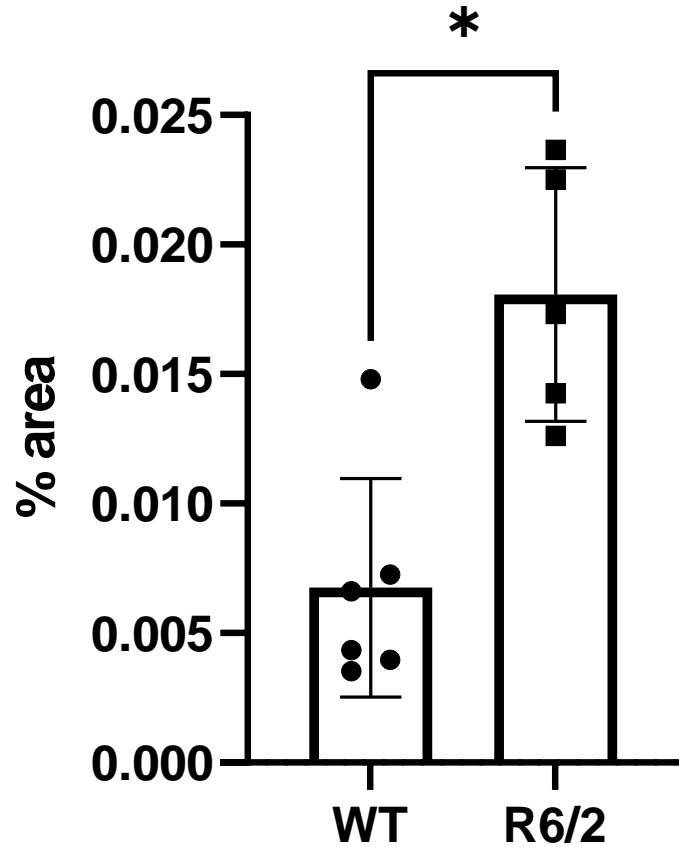
Fiber area and minimum ferret diameter in WT and R6/2 mice

● Female mice
● Male mice



Tendency to increase in fiber area and diameter in R6/2 CTX group compared to their saline group

Number of eMHC after CTX injection in WT and R6/2 mice



A trend towards increased eMHC positive cells in R6/2 compared to WT

Summary

- Both adult and neonatal R6/2 mice exhibited reduced body weight
- In H&E staining, we observed prominent gap and pattern between fibers in both adult and neonatal R6/2 muscle compared to WT muscle.
- Fiber areas were unchanged in WT mice after cardiotoxin injection but tendency to increase in R6/2 mice
- Tendency to increase in myosin heavy chain positive cell in R6/2 mice

R6/2 skeletal muscle may have the ability to regenerate new fibers

Ongoing studies

Satellite cell
proliferation capacity



Pax7 positive cells

Skeletal muscle regeneration in R6/2 mice

Inflammatory
response



CD68 and CD11b