Satellite cells fail to contribute to muscle repair but are functional in Pompe disease (glycogenosis type II)

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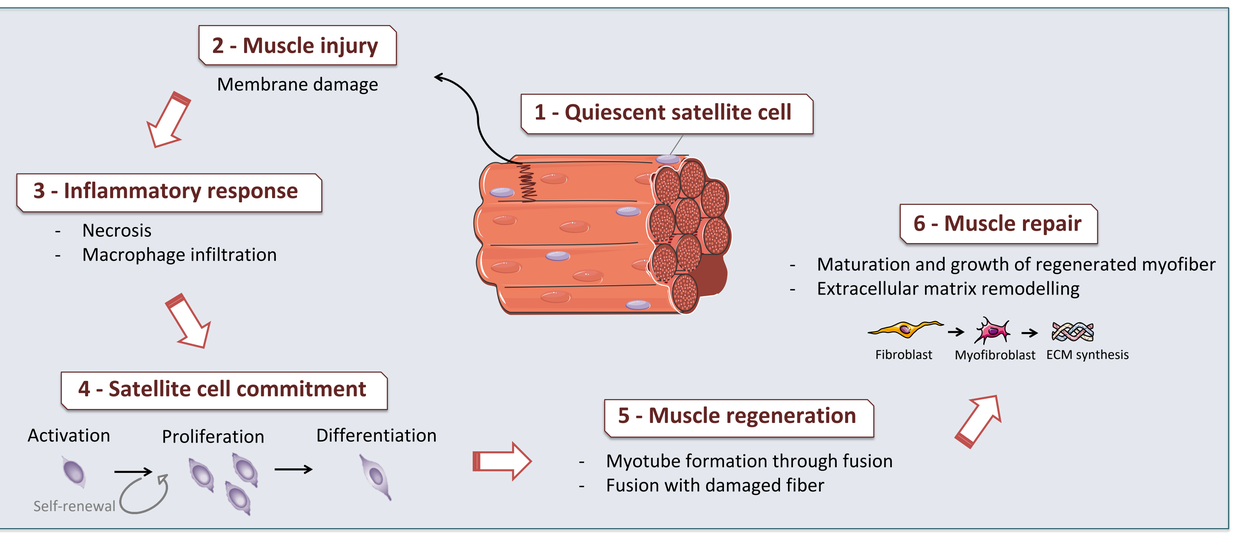
Background & Objectives

Pompe disease is a lysosomal storage disorder due to acid alpha-glucosidase (GAA) deficiency, leading to glycogen storage within lysosomes. Recently, a defect of satellite cell activation has been demonstrated on patient muscle biopsies as well as a severe alteration in the regenerative response, despite the extensive muscle damages (Schaaf *et al*, 2015).

The aim of this study was to determine the causes of the absence of satellite cells participation to muscle repair in Pompe disease and to evaluate :

1/ what is the incidence of tissue environment on satellite cell behavior in Pompe disease?

2/ what is the intrinsic potential of satellite cells in Pompe disease ?



Animals & Methods

➢ **Longitudinal histopathological study of muscle from Pompe disease mouse model**

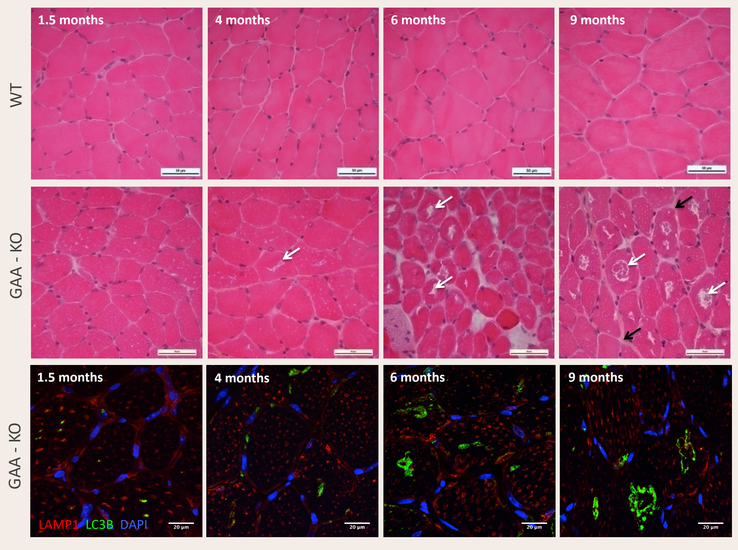
- GAA-KO mice and Wild Type (WT) aged from 1.5 to 9 months (n=5 per group)
- Hematoxylin eosin safran (HES) staining, immunolabelling against the lysosomal marker LAMP1, the autophagosomal marker LC3B and developmental myosin heavy chain isoform (MHCd)
- Phenotypic analysis of satellite cells by Pax7, Ki67, MyoD and MyoG immunolabelling

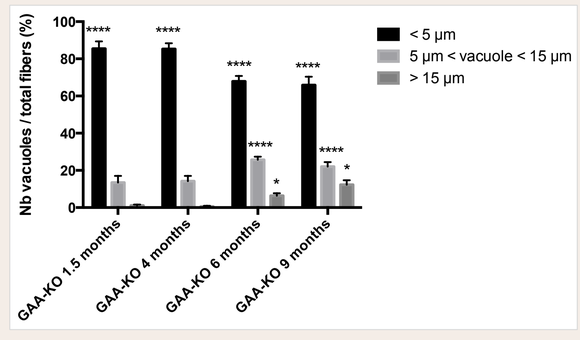
➢ **Study of muscle regeneration following injury with myotoxic agent**

- Induction of muscle injury by intramuscular injection of cardiotoxin (CTX). Euthanasia at 4, 7 and 21 days post-injection (n=4 per group)
- HES staining, immunolabelling against Pax7 and dystrophin
- Anisocytosis evaluation performed by minimum of Feret's diameter (MinFeret) measurement using Fiji software.

Results

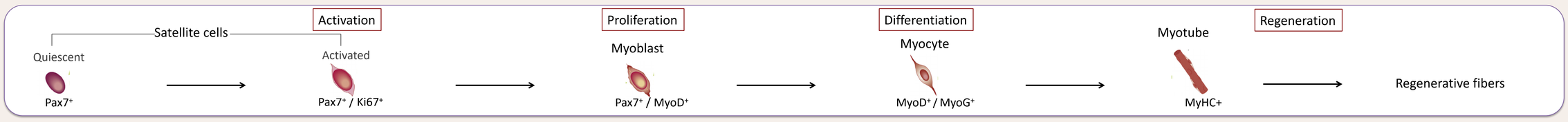
1/ Early and progressive muscle lesions related to accumulation of lysosomal and autophagic vesicles

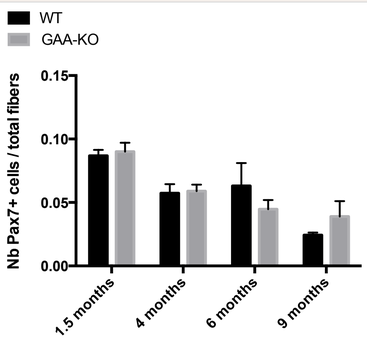




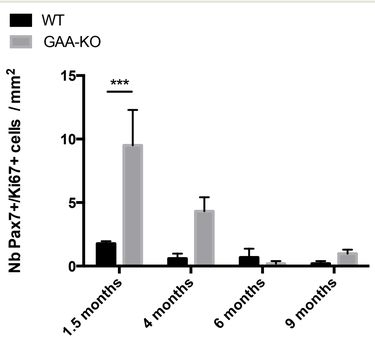
- Vacuolization of muscle fibers increasing in size and number with age of GAA-KO mice (white arrows & histogram)
- Endomysium thickening suggesting an abnormal regeneration process (black arrows)
- Progressive cytoplasmic accumulation of numerous enlarged lysosomes (LAMP1, red) and autophagosomes (LC3B, green)

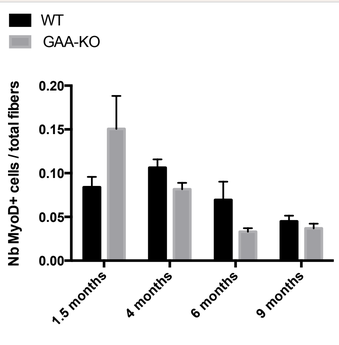
2/ Inappropriate participation of satellite cells to repair massive damaged muscle

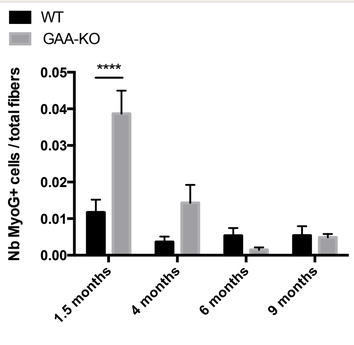




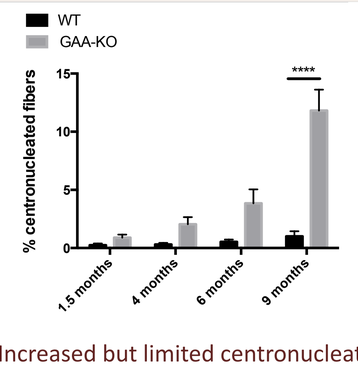
Intact satellite cell pool in GAA-KO mice muscle



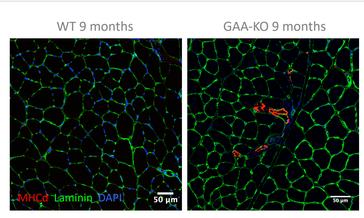




Loss of activation, proliferation and differentiation potential of satellite cells from the age of 4 months



Increased but limited centronucleation at the advanced stage of the disease



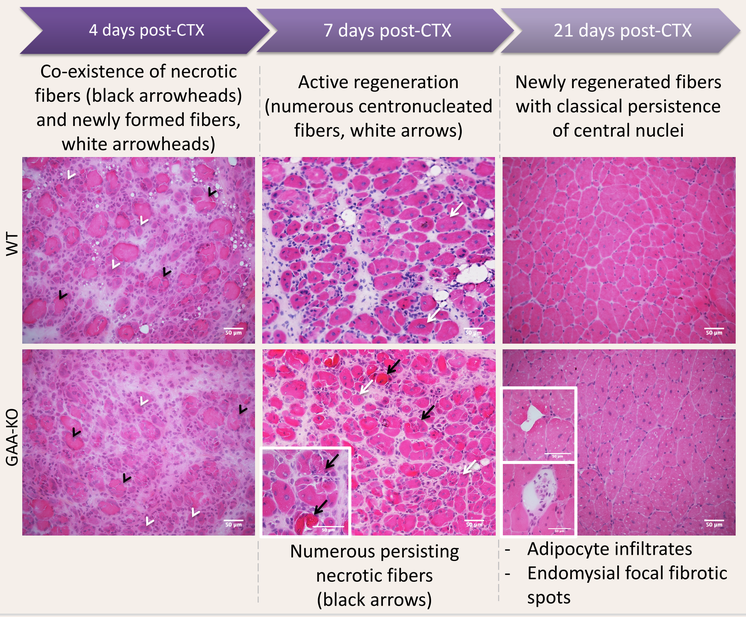
Percentage of MHCd+ fibers

WT 9 months	GAA-KO 9 months
0.15 % ± 0.03	1.30 % ± 0.51

Few small clusters of MHCd+ fibers in the whole tissue section

From age of 4 months, blocking of satellite cells in non-activated state, resulting in sporadic events of regeneration that strongly contrast with the generalized lesional state of muscle fibers

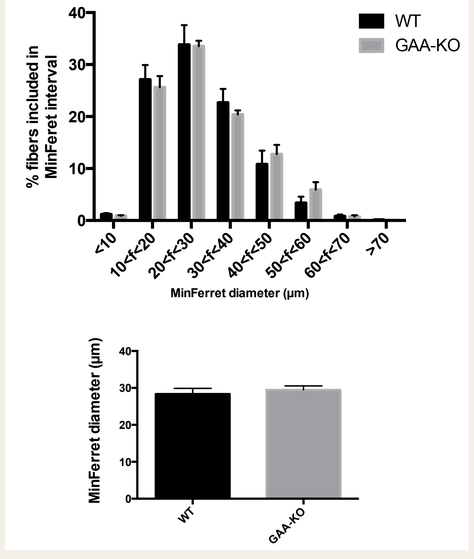
3/ Conservation of the regenerative potential for the satellite cells when submitted to acute stimulation…



In GAA-KO mice:

- Delay in the regeneration process, particularly noticeable at 7 days post-CTX
- At 21 days post-CTX, GAA-KO muscle tissue shows accomplished episode of regeneration with nevertheless sporadic histological abnormalities

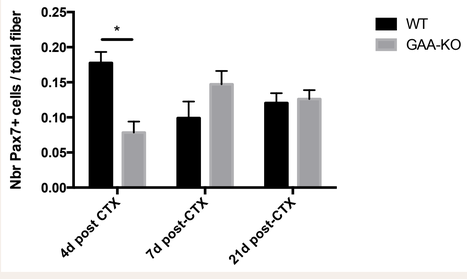
Anisocytosis evaluation at 21 days post-CTX



Homogeneous distribution of fiber size between WT and GAA-KO mice muscle

Similar mean diameter of muscle fibers between WT and GAA-KO mice

Satellite cell activation



Delayed activation in GAA-KO mice muscle

…But with a delay and muscle lesions evoking an intrinsic defect of GAA-KO satellite cells

Conclusion

- **6neo/6neo mice model** is **representative** of **human Pompe muscle**: our results are consistent with litterature regarding the satellite cell activation defect despite the huge muscle damages (Schaaf *et al*, 2015).
- **In Pompe disease**, **skeletal muscle retains regenerative potential** following *in vivo* injury, demonstrating that the **failure of SC participation** in repair **is related to** an **activation signal defect**. *In vitro* experiments and a metabolic study could be useful to better characterize this defect.
- Our findings provide **new insight into the pathophysiology of Pompe disease** and highlight that the activation signal defect of SCs compromises muscle repair, which could be related to the **abnormal energetic supply** following **autophagic flux impairment**.

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