Myopathic and neuronal pathologies of the 6^{neo} mouse model of Pompe Disease

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BACKGROUND

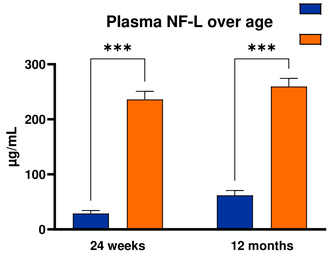
Pompe disease, a severe metabolic myopathy, is caused by mutations in the gene coding for acid α-glucosidase (GAA), the enzyme that breaks down lysosomal glycogen. Deficiency of the enzyme leads to lysosomal accumulation of glycogen in multiple tissues, including the central nervous system and muscles, resulting in a severely compromised activity. In the current study, we histologically and biochemically assessed neural and muscular disease specific features in homozygous Pompe 6^{neo} mice, a mouse model with a targeted interruption of the *GAA* gene.

MATERIALS and METHODS

6^{neo} mice of mixed sex and wild type (WT) littermates at the ages of 4, 8 and 24 weeks were included in the cross-sectional analysis. Brain and quadriceps were analyzed for pathology such as reduced GAA activity using the 4-MUG-based activity assay, and glycogen accumulation by a colorimetric glycogen assay and periodic acid schiff (PAS) staining. Quadriceps were immunofluorescently labeled for the ionized calcium binding adaptor molecule 1 (Iba1), lysosome-associated membrane protein 2 (LAMP2) and light chain 3 beta (LC3-B). Plasma was evaluated for neurofilament light chain (NF-L) levels using a commercially available kit (Uman).

RESULTS

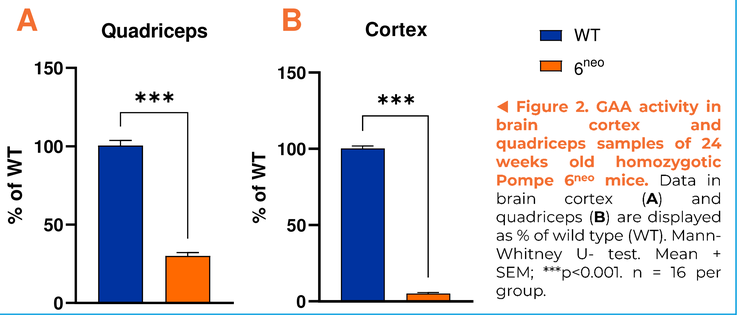
Neurofilament Light Chain in Plasma



◄ **Figure 1. Quantification of neurofilament light chain levels (NF-L) in the plasma of Pompe 6^{neo} mice.** NF-L levels in µg/mL in the plasma of 6^{neo} and WT animals at 24 weeks and 12 months of age. Two-way ANOVA with Šidák's comparison test; mean + SEM; ***p<0.001. n = 10 per group.

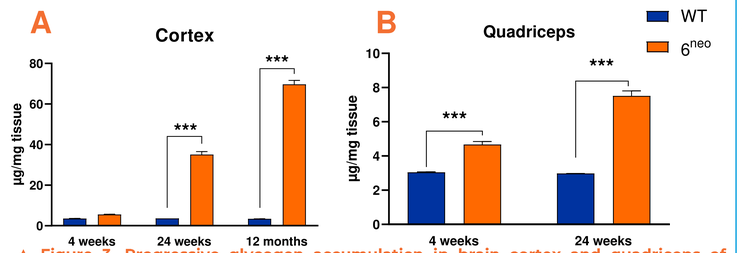
RESULTS

Reduced GAA Activity

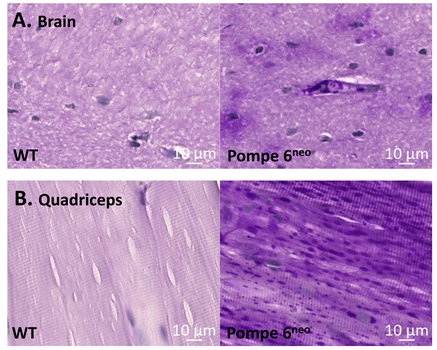


◄ **Figure 2. GAA activity in brain cortex and quadriceps samples of 24 weeks old homozygotic Pompe 6^{neo} mice.** Data in brain cortex (**A**) and quadriceps (**B**) are displayed as % of wild type (WT). Mann-Whitney U- test. Mean + SEM; ***p<0.001. n = 16 per group.

Glycogen Accumulation



▲ **Figure 3. Progressive glycogen accumulation in brain cortex and quadriceps of Pompe 6^{neo} mice.** Glycogen in tissue as µg/mg in brain cortex (**A**) of Pompe 6^{neo} and WT littermates at ages of 4, 24 weeks and 12 months, and quadriceps (**B**) at ages of 4 and 24 weeks. Two-way ANOVA with Šidák's multiple comparison test; mean + SEM; ***p<0.001. n = 16 per group.



◄ **Figure 4 . Periodic acid schiff (PAS) staining.** Representative images of PAS staining in brain (**B**) and quadriceps (**A**) of WT (**left**) and Pompe 6^{neo} (**right**) mice.

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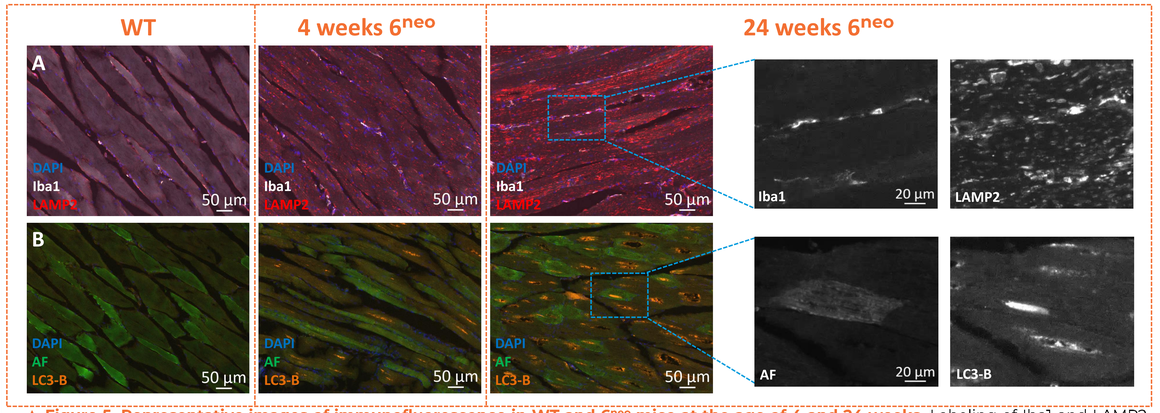
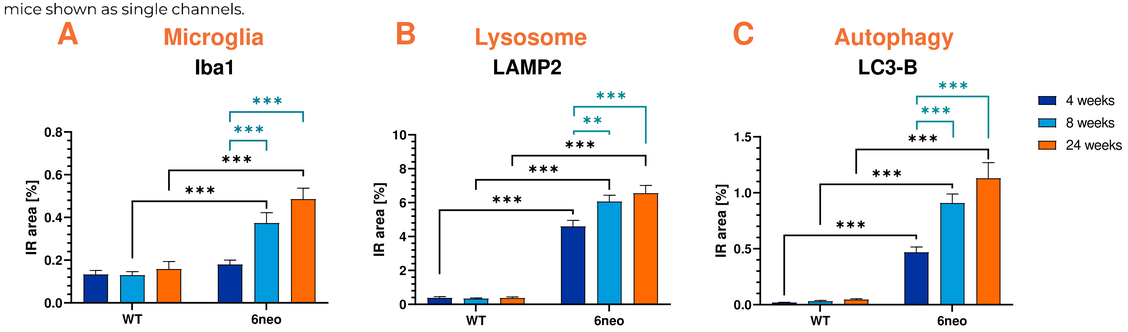
Histological Analysis of Muscle Demonstrates Progressive Pathology



▲ **Figure 5. Representative images of immunofluorescence in WT and 6^{neo} mice at the age of 4 and 24 weeks.** Labeling of Iba1 and LAMP2 (**A**), as well as LC3-B (**B**) in quadriceps of 24 weeks old WT as well as 4 and 24 weeks old Pompe 6^{neo} mice. Magnifications of the 24 weeks old 6^{neo} mice shown as single channels.



▲ **Figure 6. Quantitative analyses of immunofluorescent labeling of Iba1, LAMP2 and LC3-B in the quadriceps of 6^{neo} and WT mice with age.** Iba1 (**A**), LAMP2 (**B**) and LC3-B (**C**) immunoreactive area (IR) in percent in quadriceps of 6^{neo} mice at the ages of 4, 8 and 24 weeks and the WT littermates. Two-way ANOVA with Bonferroni's *post hoc* test; mean + SEM; ***p<0.001, **p<0.01, ***p<0.001. n = 9-12 per group.

SUMMARY and CONCLUSION

Homozygous Pompe 6^{neo} mice do not only display a muscular but also a cerebral decrease of GAA activity and progressively increasing glycogen accumulations in same tissues. Increased levels of the neurodegeneration marker NF-L were found in the plasma of 24 weeks old 6^{neo} mice, reflecting neuronal loss caused by GAA deficiency. Additionally, histological labelings validate gradual myopathic inflammatory and lysosomal alterations as well as changes in autophagy in 6^{neo} mice compared with WT mice. The Pompe 6^{neo} mouse model is thus a valuable tool for basic research and drug development against this lysosomal storage disease.