Motor Deficits in Homozygous 6^{neo} Mice as Model of Pompe Disease

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BACKGROUND

Pompe disease is an inherited lysosomal storage disease caused by a deficiency of α-glucosidase, encoded by the GAA gene. Lysosomal glycogen accumulates in tissues, including the central nervous system and muscles, most notably skeletal and cardiac muscles. In this study, we evaluated GAA knockout mice - commonly known as Pompe 6^{neo} mice - for their behavioral deficits.

While this model is over 20 years old and well characterized, reports regarding the onset of symptoms are conflicting. We therefore performed an in-depth behavioral characterization of this model.

MATERIALS and METHODS

For this study, 96 GAA knockout mice (Pompe 6^{neo}) of mixed sex and 96 wild type (WT) littermates at the age of 4, 8 and 24 weeks were included in the cross-sectional experiment. Animals were evaluated in a behavioral test battery including Irwin test, open field, RotaRod, beam walk, grip strength, and pasta gnawing test. In addition, half of animals of the 24 weeks age group were kept until the age of 52 weeks and retested in RotaRod, contextual fear conditioning and Y-maze test. Muscle weakening was evaluated by measuring the compound muscle action potential (CMAP) at 52 weeks of age. Starting at the age of 25 weeks, animals in the survival group were checked for clinical signs.

RESULTS

Muscle Strength

Reduced muscle strength of Pompe 6^{neo} mice measured by mean grip strength started at the age of 8 weeks.

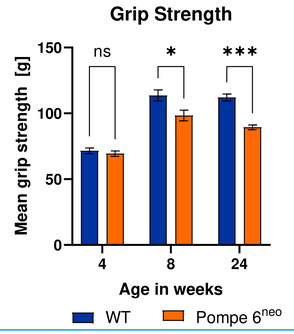

Figure 1 . Grip strength of Pompe 6^{neo} mice. Mean grip strength of 6^{neo} and WT littermates at the age of 4, 8 and 24 weeks. n = 22-48 per group. Mixed-effects analysis with Bonferroni's *post hoc* test; mean + SEM; *<0.05; ***p<0.001.

RESULTS

Motor Deficits

Evaluation of Pompe 6^{neo} mice for motor deficits in the RotaRod test resulted in first significant reductions in average latency to fall compared to WT littermates at the age of 52 weeks (Fig. 2A). Further analysis of Pompe 6^{neo} mice in the beam walk test showed motor deficits already at the age of 24 weeks (Fig. 2B). Differences between tests depend most likely on the higher sensitivity of the beam walk test.

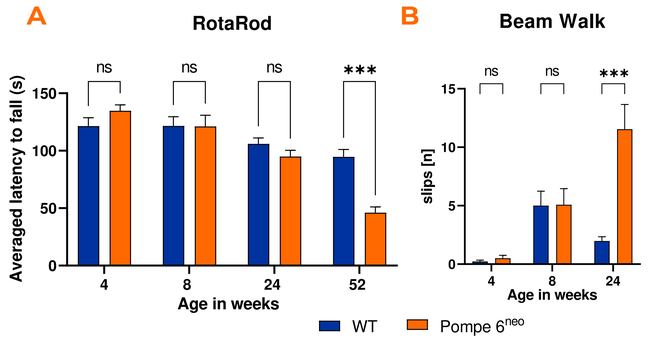

Figure 2. RotaRod and beam walk test of Pompe 6^{neo} mice. Latency to fall off the RotaRod of 6^{neo} and WT littermates at the age of 4 to 52 weeks, n = 19-48 per group. (A) Number of slips in the beam walk test of 4 to 24 weeks old 6^{neo} mice compared to WT littermates, n = 22-48 per group (B) Two-way ANOVA with Bonferroni's *post hoc* test; mean + SEM; *** p<0.001.

No significant differences were detected in contextual fear conditioning, Y-maze, Irwin test, open field and the pasta gnawing tests (data not shown).

SUMMARY and CONCLUSION

Our results show that the muscle strength of Pompe 6^{neo} is reduced already at the age of 8 weeks resulting in motor deficits that progresses with age. Impairment in neuromuscular function was also detected in 52-weeks old animals. The Pompe 6^{neo} mice model is thus a valuable tool to evaluate new compounds against this devastating lysosomal storage disease.

Muscle weakening

Electromyography recordings revealed several parameters that are indicative of impaired neuromuscular function, to be altered in 52-weeks old 6^{neo} mice. Analysis of the EMG recording revealed a decreased amplitude for CMAP initial activation (Fig. 3A), as well as a decreased CMAP amplitude (Fig. 3B) in 6^{neo} mice compared to WT mice. The latency to peak (Fig. 3C) and the CMAP duration (Fig. 3D) were significantly decreased in 6^{neo} mice.

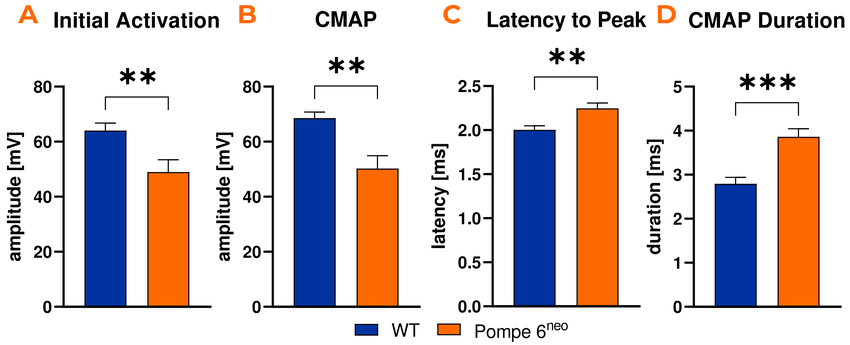

Figure 3. EMG recordings in 52-weeks old 6^{neo} and WT mice. Initial activation (A), CMAP (B), latency to peak (C), and CMAP duration (D). n = 13-14 per group. Unpaired t-test or Mann-Whitney U-test; mean + SEM; **<0.01; ***p<0.001.

Progression of clinical signs

Starting at the age of 25 weeks until the end of the study, animals of the survival group were checked daily for clinical signs. The clinical signs were used for the monthly clinical score calculation. Significant differences were observed in the clinical score, starting at the age of 33 weeks.

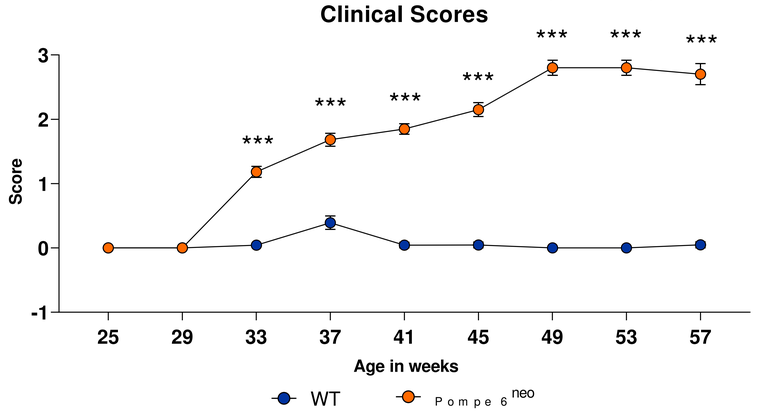

Figure 4. Clinical score in 6^{neo} mice. The clinical score was calculated based on the clinical signs that were evaluated daily. Clinical signs and scores were evaluated from the age of 25 weeks until the end of the study (57-weeks-old). n = 24 per group. Mixed-effects analysis with Bonferroni's *post hoc* test; mean ± SEM; *** p<0.001.