Data input form: MouseAGE Database

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Date submitted:

Preferred acknowledgement:

Summarise supporting data attached:

Ageing / disease: Accelerated Ageing, Osteoporosis

Model:

- PolgA$^{D257A/D257A}$ (B6.129S7(Cg)-Polg<tm1Prol>/J, Jackson Stock No. 017341)

mouse strain expressing an exonuclease-deficient version of the mitochondrial DNA polymerase γ [1]

Clinically relevant? (Yes or No):

Yes

Explanation of why clinically relevant or not:

Mutations in mitochondrial DNA (mtDNA) accumulate in tissues of mammalian species and have been hypothesized to contribute to ageing. It has been shown that the PolgA mouse model accumulates mtDNA mutations and thus develops multiple ageing phenotypes early in life [1, 2]. However, it still remains unclear whether the observed phenotypes are indeed accelerated ageing or not (reviewed in [3]).

Characteristics, timing of appearance of phenotype and phenotypic tests used (attach supporting data when unpublished if available):

- **Breeding considerations:**

As both heterozygous (PolgA$^{D257A/+}$) and homozygous (PolgA$^{D257A/D257A}$) mice have progressive accumulation of mitochondrial DNA point mutations, specific breeding considerations were taken into account while expanding the colony to obtain sufficient (female) mice for the planned experiments. More specifically, as paternal mitochondrial DNA is actively eliminated following fertilization in mice, the (undesirable) accumulation of mutations in the germline was minimized by mating heterozygous male mice (PolgA$^{D257A/+}$, B6.129S7(Cg)-Polg^{tm1Prol}/J, JAX stock #017341, The Jackson Laboratory) with C57Bl/6J inbred females (Charles River Laboratories). The thus obtained heterozygous (PolgA$^{D257A/+}$) females and males (originating from a WT C57BL/6J mother) were crossed to generate homozygous (PolgA$^{D257A/D257A}$) and WT littermates (PolgA$^{+/+}$) with only a single generation of mutation burden. Furthermore, only mice from the first litters were used for subsequent matings

and experiments, respectively. The presence of the PolgA knock-in mutation was confirmed by extracting DNA from ear clips (KAPA Express Extract, Sigma-Aldrich) followed by qPCR (SsoAdvanced Universal SYBR Green Supermix, Bio-Rad) and melt curve analysis. The primers used for genotyping were recommended by the Jackson Laboratory.

- **Characterization of bone phenotype using longitudinal micro-CT**

To characterize the bone degradation and osteoporotic phenotype in the PolgA^{D257A} mouse model, the 6th caudal vertebra of female homozygous (PolgA$^{D257A/D257A}$) and WT littermate (PolgA$^{+/+}$) controls was monitored by *in vivo* µCT (vivaCT 40, Scanco Medical) every 2 weeks between the age of 20 and 46 weeks and 3D static (AVD, length, Ct.Th, Tt.Ar, Ct.Ar/Tt.Ar., BV/TV, Tb.Th, Tb.Sp, Tb.N) and dynamic bone morphometric parameters (BFR/BRR, MS/ES, MAR/MRR) were assessed [4]. Additionally, frailty was quantified 1x/month based on the clinical mouse frailty index (FI) [5] and forelimb grip-strength was assessed using a grip-strength meter (1x/month).

In collaboration with other research groups at ETH Zurich, muscle weights and strength were measured (group of Prof. Dr. Katrien de Bock) and histology was performed on multiple organs (heart, liver, skin …) dissected from mice of different ages.

If applicable, Intervention(s) performed (type, dose, route of administration, frequency)

- **Effect of mechanical loading on bone remodeling**

To investigate the effects of cyclic mechanical loading of the 6th caudal vertebra in the PolgA^{D257A} mouse model, female, 38 week-old, PolgA$^{D257A/D257A}$ (n=10) and PolgA$^{+/+}$ (n=10) were loaded (3x/week, 8N, 10Hz) over a period of 4 weeks with weekly *in vivo* µCT monitoring and compared to non-loaded controls (n=10/group). As explained above, 3D static and dynamic bone morphometric parameters were assessed. Frailty was quantified weekly by measuring FI and forelimb grip-strength was assessed using a grip-strength meter at the beginning (age 38 weeks) and end of the study (age 42 weeks).

Phenotype post-treatment (include null or negative results and attach supporting data separately):

The data has not yet been fully evaluated but can be made available in full after publication of the basic findings.

References (list below):

1. Kujoth, G.C., et al., *Mitochondrial DNA Mutations, Oxidative Stress, and Apoptosis in Mammalian Aging.* Science, 2005. **309**(5733): p. 481.
2. Trifunovic, A., et al., *Premature ageing in mice expressing defective mitochondrial DNA polymerase.* Nature, 2004. **429**(6990): p. 417-23.
3. Kõks, S., et al., *Mouse models of ageing and their relevance to disease.* Mechanisms of Ageing and Development, 2016. **160**: p. 41-53.
4. Lambers, F.M., et al., *Mouse tail vertebrae adapt to cyclic mechanical loading by increasing bone formation rate and decreasing bone resorption rate as shown by time-lapsed in vivo imaging of dynamic bone morphometry.* Bone, 2011. **49**(6): p. 1340-50.

5. Whitehead, J.C., et al., *A Clinical Frailty Index in Aging Mice: Comparisons With Frailty Index Data in Humans.* Journals of Gerontology Series a-Biological Sciences and Medical Sciences, 2014. **69**(6): p. 621-632.