DELAYED SKELETAL MUSCLE REGENERATION IN AN ACCELERATED AGEING MOUSE MODEL.

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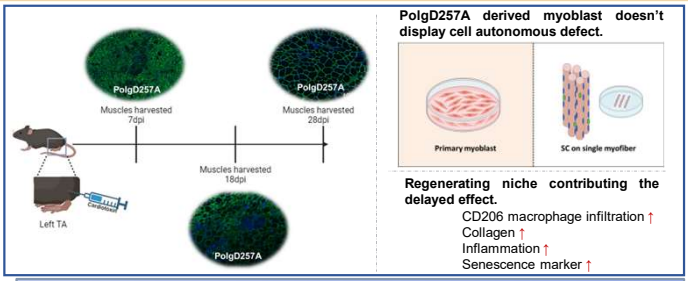
❖ Background:

- **PolgD257A Mouse Model:** PolgD257A knock-in mice exhibit premature aging symptoms, including anemia, alopecia, and sarcopenia, due to impaired DNA-proofreading activity of Polg, leading to mitochondrial dysfunction and elevated DNA mutations (Kujoth 2005; science).
- **Sarcopenia, Mitochondrial Myopathy, and Muscle Regeneration:** Sarcopenia, characterized by muscle loss with aging, and primary mitochondrial myopathy, where mitochondrial DNA mutations lead to muscle weakness, both contribute to defective muscle regeneration. In PolgD257A mice, these mitochondrial defects impair muscle repair, highlighting the critical role of mitochondrial function in regeneration.
- **Inflammaging and Senescence:** The persistent inflammation (inflammaging) and accumulation of senescent cells in the aged muscle microenvironment further hinder proper muscle regeneration in PolgD257A mice.

❖ Aim of the project:

This study aims to develop a model using PolgD257A mice that assesses age-related muscle regeneration defects and investigates how mtDNA mutation accumulation and mitochondrial dysfunction contribute to impaired muscle repair during aging.



PolgD257A derived myoblast doesn't display cell autonomous defect.

Regenerating niche contributing the delayed effect.

CD206 macrophage infiltration ↑
Collagen ↑
Inflammation ↑
Senescence marker ↑

1) PolgD257A mice exhibit sarcopenic features.

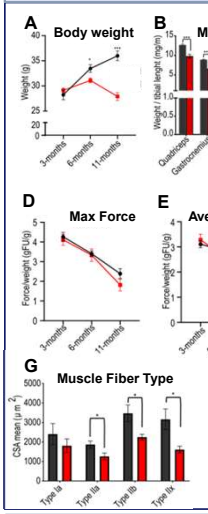


Fig.1. Characterization of sarcopenic features between Wild type vs PolgD257A. (A) Body weight at 3,6 and 11-months in grams.; (B) Muscle weight quadriceps, gastrocnemius, soleus, EDL, tibialis anterior and Diaphragm in mg. (n≥6); (C) Fats weights such as SCF, IAT, BAT and Ep Fat. and (D) Average force (E)Max Force normalised over body weight in gFU/g (n≥6); and (F) Representative fluorescent picture of muscle fiber type Blue: Type Ia, Green: Type IIa and Red: IIb and (G) Average CSA of muscle fiber type. (n≥4). *P<0.05, **P<0.01, ***P<0.001 Multiple t test.

2) PolgD257A mice exhibit delayed muscle regeneration at both 3- and 11-months age.

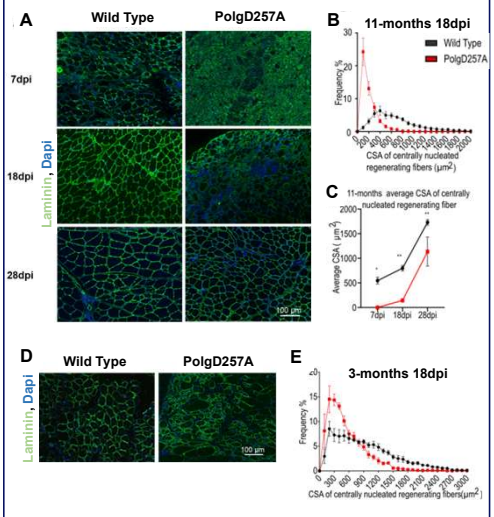


Fig. 2. Delayed Muscle Regeneration in PolgD257A. (A) Representative pictures of transverse sections of TA in WT and PolgD257A at 11 months of age stained with antibodies against laminin (green) and DAPI (blue) at 7dpi, 18dpi, and 28dpi (Top panel: 7dpi, middle panel: 18dpi, and bottom panel: 28dpi). (n≥3); **(B)** Percentage of CSA frequency distribution of centrally nucleated myofibers in TA at 18dpi of 11-month-old WT and PolgD257A (n=6); **(C)** Average CSA of centrally nucleated myofibers of TA at 7dpi, 18dpi, and 28dpi in WT and PolgD257A at 11 months of age. (n≥3); **(D)** Representative picture of transverse section of TA in WT and PolgD257A at 3 months of age stained with antibodies against laminin (green) and DAPI (blue) at 7dpi, and **(E)** Percentage of CSA frequency distribution of centrally nucleated myofibers in TA at 7dpi of 3-month-old WT and PolgD257A (n=6). Scale bar: 100 µm. (*P<0.5).

3) Reduced SC Population and MRF Gene Expression in Regenerating Muscle of PolgD257A Mice at 11 Months.

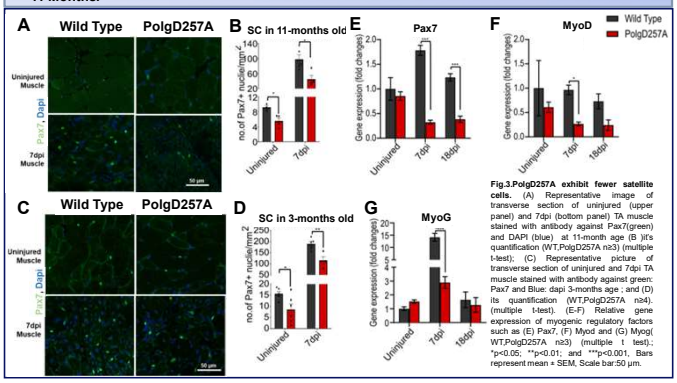


Fig.3.PolgD257A exhibit fewer satellite cells. (A) Representative image of transverse section of uninjured (upper panel) and 7dpi (bottom panel) TA muscle stained with antibody against Pax7(green) and DAPI (blue) at 11-month age (B)it's quantification (WT,PolgD257A n≥3) (multiple t-test); (C) Representative picture of transverse section of uninjured and 7dpi TA muscle stained with antibody against green: Pax7 and Blue: dapi 3-months age ; and (D) Its quantification (WT,PolgD257A n≥4). (multiple t-test). (E-F) Relative gene expression of myogenic regulatory factors such as (E) Pax7, (F) Myod and (G) Myog(WT,PolgD257A n≥3) (multiple t test).; *p<0.05; **p<0.01; and ***p<0.001, Bars represent mean ± SEM, Scale bar:50 µm.

4) PolgD257A derived myoblast doesn't display cell autonomous defect.

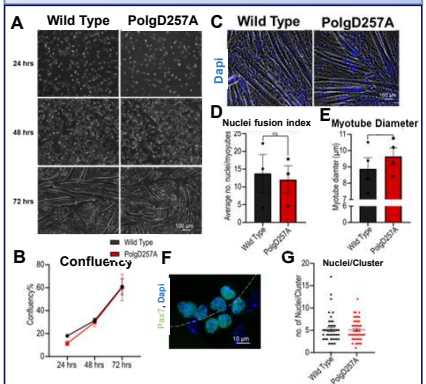


Fig.4.Primary in vitro cell culture.(A) Brightfield images of primary myoblast harvested from 11-month age Wt vs PolgD257A mice cultured in vitro at 24hrs, 48hrs and 72 hours.;(B) Percentage confluency of cells at 24hrs,48hrs and 72 hrs.(Scale Bar 100 µm); (C) Representative picture of myotubes from 11-months old PolgD257A and Wild type depicting myotube diameter and nuclei fusion per myotube. . (Scale Bar 100 µm; (D) quantification of nuclei fusion index (E) quantification of myotube diameter, (G) Representative picture SC cluster on single myofiber harvested from 11-month age WT vs PolgD257A and cultured in vitro for 72 hrs; and (H) Its quantification. (Bars represent mean ± SEM, scale bar:10µm)

5) Satellite cell non-autonomous crucially influences muscle regeneration in PolgD257A mice

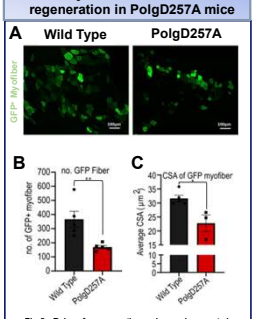


Fig.5. Role of regenerating microenvironment in lesser engraftment of WT-GFP myoblast in 11-month-old recipient PolgD257A. (A) Fluorescent image of GFP+ myofibers in transplanted TA of WT and PolgD257A; (B) Quantification of GFP+ myofiber and (C) quantification of average CSA of engrafted WT-GFP myofiber (Wild Type n=5, PolgD257A n=5; * p<0.05, **p<0.01, t-test, Bars represent mean ± SEM, scale bar: 100µm).

6) Altered macrophage dynamics and inflammatory, FAP-related gene expression in regenerating PolgD257A muscle

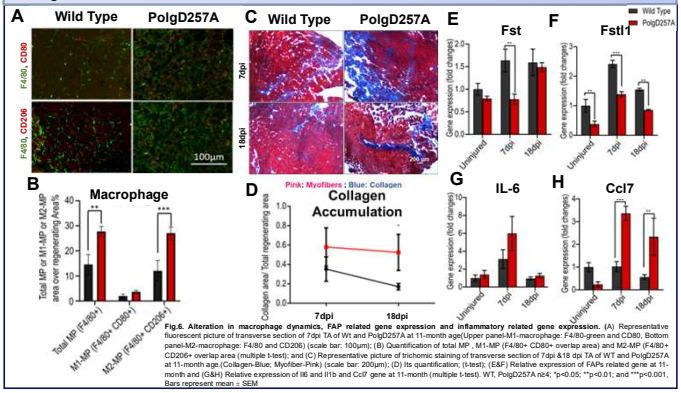


Fig.6. Alteration in macrophage dynamics, FAP related gene expression and inflammatory related gene expression. (A) Representative fluorescent picture of transverse section of 7dpi TA of Wt and PolgD257A at 11-month age(Upper panel-M1-macrophage: F4/80-green and CD80, Bottom panel-M2-macrophage: F4/80 and CD206) (scale bar: 100µm); (B) Quantification of total MP , M1-MP (F4/80+ CD80+ overlap area) and M2-MP (F4/80+ CD206+ overlap area (multiple t-test); and (C) Representative picture of trichomic staining of transverse section of 7dpi & 18 dpi TA of WT and PolgD257A at 11-month age.(Collagen-Blue; Myofiber-Pink) (scale bar: 200µm); (D) Its quantification; (t-test); (E&F) Relative expression of FAPs related gene at 11-month and (G&H) Relative expression of Il6 and Il1b and Ccl7 gene at 11-month (multiple t-test). WT, PolgD257A n≥4; *p<0.05; **p<0.01; and ***p<0.001, Bars represent mean ± SEM

7) Enhanced gene expression and histological markers of senescence in PolgD257A muscle at 7 dpi.

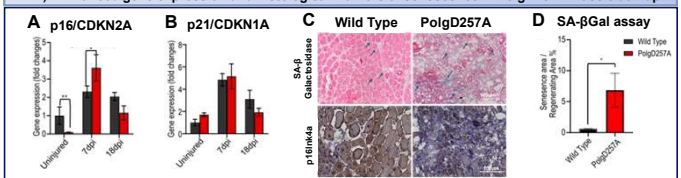


Fig.7. Altered senescence related marker in PolgD257A at 7dpi. (A and B) Relative gene expression of senescence markers, p16/CDKN2A and p21/CDKN1A. (C) Representative images of SA-βGAL staining assay (blue- senescence cells and (eosin) pink- cytoplasm) (Upper panel) and immunohistochemistry for p16Ink4a (brown) and haematoxylin-nuclie (blue) (lower panel) (scale bar 100µm) and (D) Its quantification. (multiple t-test). WT, PolgD257A n≥4; *p<0.05; **p<0.01; and ***p<0.001, Bars represent mean ± SEM