# Mitochondrial DNA Mutations in the Polymerase Gamma Mutator Mouse Increases the Severity of Traumatic Osteoarthritis

Nada H. Warda[1,3,*], Abdulkadir Nur[1], Amogh Kankanwadi[2], Madison M. Buckles[2], Huzafa Khanzada[2], Ethan Hyrne[1], Mohammad Y. Ansari[1,#]

[1]Department of Biomedical Sciences, Northeast Ohio Medical University (NEOMED), Rootstown, OH,

[2]College of Medicine, Northeast Ohio Medical University, Rootstown, OH

[3]University Hospitals Research Scholar, NEOMED, Rootstown, OH

[*]Email of presenting: nwarda@neomed.edu; [#]email of corresponding author: mansari1@neomed.edu

DISCLOSURES: The authors have nothing to disclose.

INTRODUCTION**:** All eukaryotic cells contain mitochondrial DNA (mtDNA) that has genes encoding the proteins of oxidative phosphorylation, tRNAs, and rRNAs necessary for mitochondrial gene translation. mtDNA replication and repair depend solely on DNA polymerase, Polymerase gamma (PolgA). The PolgA D257A/D257A "mutator" mouse carries a mutation in the proofreading domain, which accelerates the accumulation of mtDNA mutations, mitochondrial dysfunction, and eventually premature aging. Osteoarthritis (OA) is the most common form of arthritis among adults. Knee joints are the most affected joints, affecting functional ability and quality of life. To provide therapeutic strategies targeting mitochondria, the link between mitochondrial health in joint integrity and OA progression must be established. In the present study, we investigated the impact of mtDNA mutation on traumatic OA pathogenesis using the PolgA mutator mouse model.

METHODS**:** All animal studies were approved by NEOMED IACUC. Destabilization of the medial meniscus (DMM) surgery was performed on 12-week-old wild-type (WT) and PolgA mutant mice. Sham surgery was done as a control. The distribution included DMM WT, which consisted of 16 mice (8 males, 8 females), and the DMM knock-out (KO) group included 15 mice (8 males,7 females). The sham group was 10 WT mice (5 males, 5 females), and the sham KO group included 10 mice (5 males, 5 females). Pressure application measurement (PAM) for knee hyperalgesia was recorded before surgery as a baseline, followed by biweekly data collection after surgery. At 8 weeks post-surgery, the mice were euthanized, the knee joints were dissected, and fixed in 10% neutral buffered formalin. Scans were performed using micro-computed tomography (Micro-CT) with a SkyScan 1273 micro-CT scanner. Parameters used were 90 kV, 166 µA current, and 10 µm pixel resolution scanning. Images were reconstructed with Nrecon software and analyzed for 3D view using Amira-Avizo and CTan software. Regions of interest (ROI) were selected to assess trabecular bone parameters, including bone volume fraction (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp). In addition, bone mineral density (BMD), total tissue volume, osteophyte formation, and patellar thickness were quantified. After micro-CT, we performed histological evaluation of the knee joints using H&E, Toluidine Blue-Fast green, and Picro Sirius red staining. The severity of OA was assessed by two blind observers using the OARSI scoring method. Immunostaining was performed to determine IL-6 expression, and oxidative DNA damage was determined using 8-OHdG antibody. Statistical significance was calculated using one-way ANOVA followed by Dunnett's test for post hoc analysis.

RESULTS**:** Pain assessment in the DMM knee joint using PAM showed a significant decrease in pressure threshold in PolgA mutant mice compared to age and sex matched WT. Micro-CT analysis showed clear differences in bone remodeling between WT and PolgA mutant mice. Interestingly, compared to WT, the PolgA mutant mice DMM knee joints showed an increase in osteophytes, lower bone volume and density, thinner and fewer trabeculae, and more trabecular separation, indicating altered bone remodeling in the PolgA mutant mice. Compared to WT, histological analysis of the DMM knee joint (Figure 1) revealed higher cartilage extracellular matrix loss, lower chondrocyte density, higher subchondral bone remodeling, lesions in the meniscus and synovial membrane in the PolgA mutants. Immunostaining demonstrated high IL-6 levels and oxidative DNA damage in mutant joints, suggesting increased inflammation and oxidative stress in the PolgA mutant joints subjected to DMM surgery. Furthermore, OARSI scoring demonstrated a significantly higher disease severity in PolgA mutant mice compared to wt mice. These results show that accumulation of mtDNA mutations leads to increased severity of OA in PolgA mutant mice.

CONCLUSION**:** Accumulation of mtDNA mutations in PolgA mutator mice led to increased oxidative stress and inflammation, which further exacerbates OA-related subchondral bone changes and cartilage degeneration following joint injury.

ACKNOWLEDGEMENTS. This project is supported by funds from NEOMED to MYA.

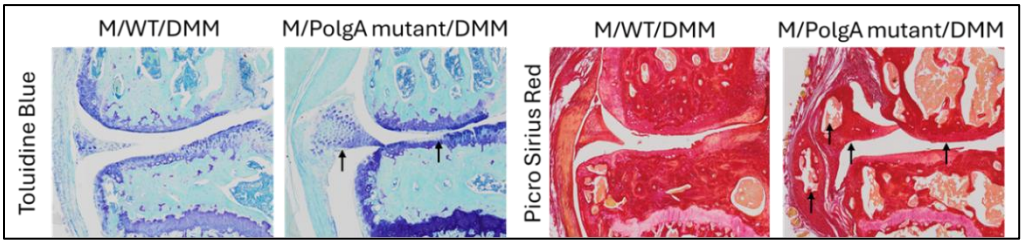


Figure 1. Representative images of Toluidine Blue-Fast green and Picro Sirius Red staining of WT and PolgA mutant knee joints subjected to DMM surgery.