Age- and Sex-Dependent Bone Phenotype and Severe Spontaneous Osteoarthritis in Atp13a2 Knockout Mice

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INTRODUCTION. Atp13a2 is a lysosomal P-type ATPase that plays a role in keeping normal lysosomal and mitochondrial function by regulating divalent cations and amines. Global deletion of Atp13a2 results in lysosomal dysfunction, including impaired autophagy and reduced activity of lysosomal enzymes. Osteoblasts and osteoclasts are necessary for bone remodeling. Cathepsin K and tartrate-resistant acid phosphatase in osteoclasts are key enzymes in bone resorption and are activated in lysosomes. Atp13a2 regulates zinc and manganese concentration in the cytoplasm, protects mitochondria from zinc-originated oxidative stress and provides manganese for enzymes to work properly, which produce collagen 1 to make more bone. Osteoarthritis (OA) is a degenerative joint disease, and cartilage loss is a hallmark of advanced OA. In this study, we hypothesize that deletion of Atp13a2 impairs lysosomal function, disrupts autophagy, induces oxidative stress, impairs both osteoblast and osteoclast activity, leading to disruption of bone remodeling and also increases chondrocyte apoptosis and matrix breakdown.

METHODS. To study the impact of Atp13a2 deficiency on bone, cartilage and overall health of the joint, spines, tails, femoral bones and knee joints were analyzed in Atp13a2 knockout mice. Samples were harvested at 3- and 18-months of age from male and female mice (n=9 per age group). To study bone phenotype, micro-computed tomography (micro-CT) was used to scan bone samples, and CTAn software was used to analyze bone volume, trabecular thickness, trabecular number, and trabecular separation. Histology and immunostaining studies were performed to explore bone and joint morphology and related target proteins. The femoral heads from WT and Atp13a2 KO mice were stained with LysoSensor, followed by imaging to assess lysosomal function. All data were tested for normality by the D'Agostino & Pearson test and Shapiro-Wilk test, then statistical significance between the two groups was calculated using a two-tailed, unpaired t-test with Welch's correction if the data passed the normality test; if the Data failed the normality test, then statistical significance between groups was calculated using the Mann–Whitney U-test.

RESULTS. Bone micro-CT analysis displayed sex and time-dependent significant differences between KO and WT mice. In the epiphysis, bone volume to tissue volume, trabecular thickness, trabecular number, significantly decreased, and trabecular separation significantly increased in 3-month-old female Atp13a2 KO mice compared to WT, but there was no difference in male (Figure 1). There were similar results in the diaphysis. Bone mineral density was significantly decreased in both 3-months and 18-month-old female KO mice compared to the corresponding WT group. Cartilage thickness was reduced, and chondrocyte numbers were decreased in the cartilage of the femoral head. Aged Atp13a2 KO mice showed a high degree of cartilage degeneration in the knee joint with a higher OARSI score as determined by Toluidine blue staining. H&E staining revealed reduced chondrocyte density in the aged Atp13a2 KO mice compared to wild-type littermates. Micro-CT imaging showed a higher osteophyte formation in the Atp13a2 KO mice`s knees compared to WT littermate controls.

DISCUSSION. Mice deficient in Atp13a2 displayed a bone phenotype that progressed with age and varied by sex. Our findings also showed that the loss of Atp13a2 function accelerated oxidative stress and apoptosis in chondrocytes, which increased osteoarthritis severity in aging mice.

SIGNIFICANCE. The current study is the first to report a bone phenotype in mice lacking Atp13a2 and highlights the importance of lysosomes in bone remodeling and osteoarthritis pathogenesis.

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Figure 1

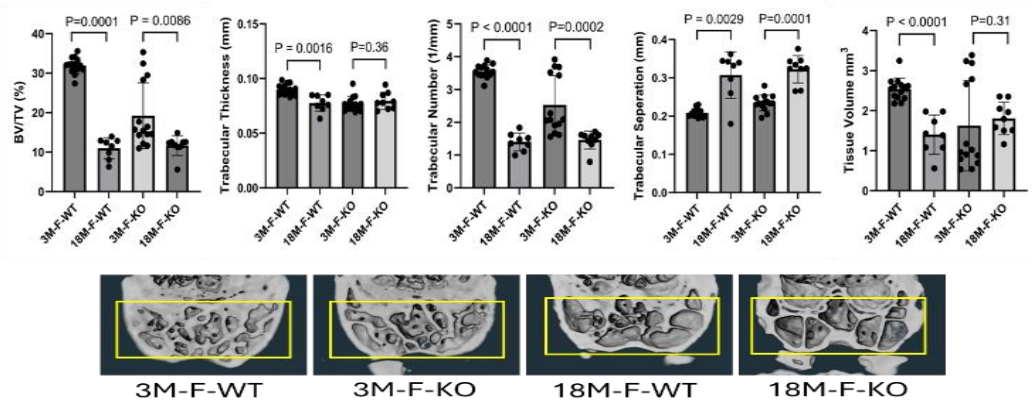

Figure 1: Morphometric analysis of epiphysis of femoral bone.