# Articular cartilage mechanical properties do not differ between C57BL/6J and MRL/MpJ mouse strains

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**INTRODUCTION**: Osteoarthritis (OA) is a pervasive joint disease affecting over 50 million people in the United States. One of the main complicating factors with OA pathology is that articular cartilage is a tissue with a poor ability to regenerate following injury or pathologic degradation. For many years, mice have been a crucial model used to study OA progression including the degradation of articular cartilage. The Murphy Roths large (MRL/MpJ) mouse strain was incidentally discovered to have "superhealing" abilities including the ability to repair ear punctures. Recent studies have also shown that MRL mice are resistant to articular cartilage degradation following intra-articular fracture or non-invasive ACL rupture. It is currently unclear if this OA resistance is due to accelerated cartilage healing or resistance to cartilage degradation. In order to gain insight on the mechanism of this OA resistance, it is important to characterize the structural, compositional, and mechanical differences between articular cartilage of MRL mice and OA-vulnerable strains such as C57BL/6J (B6) mice. In this study, we characterized the mechanical properties of the articular cartilage in the knees of MRL and B6 mice using two mechanical testing methods. Namely, we used cartilage micro-indentation to map the structural stiffness on the distal femoral condyles as well as Atomic Force Microscopy (AFM)-based nanoindentation to determine the effective indentation modulus (resistance to indentation) of articular cartilage on the medial femoral condyle. We hypothesized that stiffness of the articular surface and effective indentation modulus would be greater in MRL mice, which may contribute to their OA resistance.

**METHODS:** 5 male C57BL/6J mice and 5 male MRL/MpJ mice (12-weeks-old at the time of euthanasia) were used for micro-indentation testing to determine regional stiffness of the femoral condyle articular cartilage. Femurs were affixed to a stage using cyanoacrylate in a bath of phosphate-buffered saline (PBS). Using an indenter with a 0.3 mm spherical tip under a uniaxial load cell, the mechanical tester (Mach-1 v500css, Biomomentum) was calibrated to test several locations on both the medial and lateral femoral condyles for each mouse. At each location, structural stiffness (N/mm) was obtained. Another 4 male B6 mice and 4 male MRL mice were euthanized at 28 weeks for AFM-based nanoindentation testing. Nanoindentation was conducted on the medial femoral condyle of each joint in PBS to measure the effective indentation modulus of cartilage. Locally flat locations for nanoindentation were chosen and at least 10 locations were tested at an indentation depth rate of ≈ 10 µm/s in 1× PBS with a microspherical tip ($R \approx 5$ µm, $k \approx 8.9$ N/m).

**RESULTS:** Micro-indentation testing showed no significant differences in mean stiffness of the medial femoral condyles of B6 and MRL mice, while the lateral condyles of MRL mice exhibited a trend toward higher stiffness than B6 mice (+22%, p = 0.063, Figure 1C). When medial and lateral femoral condyles were analyzed in subregions, with regions I and II making up the lateral condyle and regions III and IV making up the medial condyle (Figure 1A, B), significant subregional differences were observed between MRL and B6 mice. For example, region III of the medial condyles of MRL mice were 30% stiffer than the same region of B6 mice, while region IV showed the opposite difference, with the cartilage of B6 mice being 28% stiffer than the same region of MRL mice (Figure 1D). AFM-based nanoindentation of medial condyle articular cartilage showed no significant differences in effective indentation modulus between B6 and MRL mice (Figure 1E).

**DISCUSSION:** In this study, we examined mechanical differences in articular cartilage between OA-resistant (MRL/MpJ) and OA-vulnerable (C57BL/6J) mouse strains to determine if there is a mechanical basis for the chondroprotection of MRL mice relative to B6 mice. Despite there being no overall differences between mechanical properties of cartilage between the mouse strains, MRL and B6 mice exhibited some significantly different subregional patterns of stiffness, with the MRLs being most stiff in the lateral aspect of the medial femoral condyles and the B6s being most stiff in the medial aspect of the medial condyles. It is currently unclear if this difference in subregional stiffness is a mechanical factor that affects cartilage degeneration on the femoral condyles. Spatial transcriptomics/proteomics in future experiments may help to shed more light on whether regional differences in stiffness correlate with changes in extracellular matrix composition.

**SIGNIFICANCE/CLINICAL RELEVANCE:** This study contributes to the efforts of determining factors that contribute to the chondroprotective nature of OA-resistant (MRL/MpJ) mice. Identifying contributing factors to the OA-resistance in this mouse strain will offer insights to potential therapeutic approaches for treating and preventing OA in human patients.

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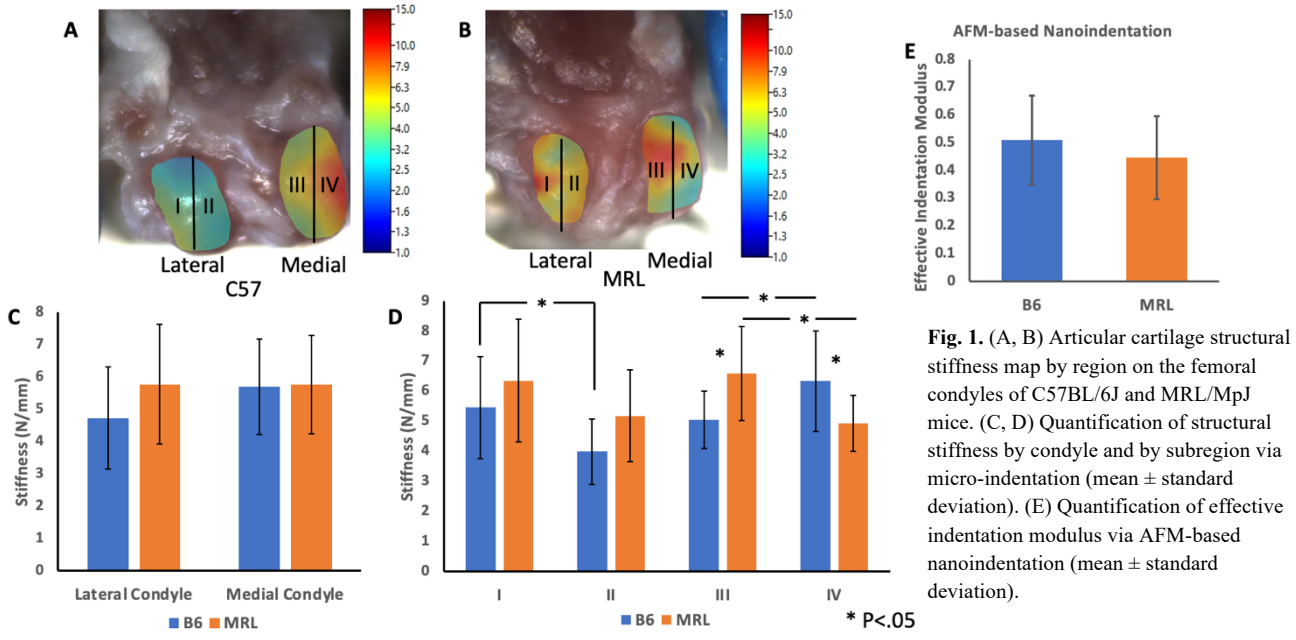


**Fig. 1.** (A, B) Articular cartilage structural stiffness map by region on the femoral condyles of C57BL/6J and MRL/MpJ mice. (C, D) Quantification of structural stiffness by condyle and by subregion via micro-indentation (mean ± standard deviation). (E) Quantification of effective indentation modulus via AFM-based nanoindentation (mean ± standard deviation).