Introduction  Osteoarthritis is a process whereby articular cartilage matrix is mechanically damaged, and its severity correlates with biochemical differences accounting for a weakened structural matrix. The constituents of the extracellular matrix (ECM) of articular hyaline cartilage include type II collagen (Col II), proteoglycans and glycosaminoglycans, and glycoproteins, including matrilins. Previous research showed that mice knees lacking matrilin-3 develop osteoarthritis, but no studies have established a link with matrilin-1. This investigation has shown that matrilin-1 knock-out (KO) mice are, post-operative for destabilization of the medial meniscus (DMM), more prone to develop osteoarthritis with mechanical deficiency than the WT mice.

Methods  DMM, approved by Rhode Island Hospital IACUC, was used to induce osteoarthritis of the knee. All DMM procedures were performed on the right leg of 6-8 week-old male mice. 9 MATN1+/- mice were assigned to the WT group. 11 MATN1-/- mice were included in the KO group. Post-op, the mice were free to move naturally.

Histological specimens of knee joints were harvested at 8 weeks post-op and stained with Safranin-O for GAG content in articular cartilage. On section, the tibial and femoral articulating surfaces defined between the two meniscuses were used as the standard area for comparison. Relying on a 6-point scoring system for mouse articular cartilage developed by the Osteoarthritis Research Society International, two members of the research team independently scored the tibial and femoral surfaces in all slides. WT and KO tibia scores and WT and KO femur scores were averaged and analyzed by a student t-test.

Atomic force microscopy (AFM) analysis was used to assess the mechanical changes in matrilin-1 KO ECM. Articular cartilage from the femoral heads was cryosectioned to obtain thin tissue sections, which were immersed in PBS with protease inhibitors. Indentation tests were conducted using a 5 μm diameter spherical probe (k~4.5 N/m). Data points were collected from 4 samples (2 groups of 1 WT, 1 KO) and analyzed for mean elastic modulus.

Real-time RT-PCR was performed to quantify mRNA levels of Col II in the femoral condyle cartilage of 10 week-old WT and KO (non-DMM) mice. Total RNA was isolated using RNAweuse--4PCR.

Results  The mean of the tibia degradation scores for KO mice was higher than the WT mice tibia scores (p<0.05). KO tibia mean score was 3.71±1.94, and WT tibia mean score was 1.82±1.23. Histologically, the KO joints are eroded more with visibly less Safranin-O staining, indicating articular surface destruction (Fig. 1). Femur scores were not statistically different.

AFM mechanical testing showed that KO cartilage had lower mechanical elasticity than WT (Fig. 2). Data points were collected from arrays of test sites at multiple locations on each specimen. The average elastic modulus of WT data points (n=800 curves) equaled 127±73 kPa, versus 76±48 kPa for the KO sample (n=800 curves), a statistically significant difference (p<0.05) by student t-test.

Discussion  Matrilin-1 KO mice had decreased expression of Col II mRNA in addition to the lack of functional matrilin-1 in cartilage. Even in cartilage without osteoarthritis (non-DMM), the absence of matrilin-1 results in decreased expression of Col II, an integral structural component of the ECM structure. The effects of matrilin-1 deficiency and Col II reduction on cartilage mechanical properties were quantifiable using AFM elastic testing. The cartilage matrix becomes less stiff compared to normal. Matrilin-1 forms filaments with collagens, especially Col II, in assembly and structure, and mutations affect its connecting properties. The complete absence of matrilin-1 coupled with down-regulation of Col II may contribute to an inherent deficiency in mechanical properties and matrix integrity of articular cartilage in the KO mice.

The functional effects of the microstructural changes are now understood by the histological analysis, which revealed an articular surface more eroded in the matrilin-1 KO mice than the WT mice. Additionally, the scores quantify the differences: a score of 4/6 (~3.71 KO) on the OARSI scale represents degradation up to 50% of the articular cartilage depth, while a score of 2/6 (~1.82 WT) describes damage to immediately below the superficial surface. The absence of matrilin-1 in ECM causes the cartilage to degrade more in the DMM model, and therefore could be a genotypic anomaly that results in an age-dependent osteoarthritis phenotype.

Significance  Matrilin-1 is an essential regulatory and protective component of articular cartilage, and its absence has manifold effects that predispose the knee to develop osteoarthritis.