Deficient Mechanical Activation of Anabolic Transcripts and Post-Traumatic Cartilage Degeneration in Matrilin-1 Knockout Mice

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Abstract

Matrilin-1 (Matn1), a cartilage-specific peri-cellular and extracellular matrix (ECM) protein, has been hypothesized to regulate ECM interactions and transmit mechanical signals in cartilage. Since Matn1 knock-out (Matn1−/−) mice exhibit a normal skeleton, its function in vivo is unclear. In this study, we found that the anabolic Acan and Col2a transcript levels were significantly higher in wildtype (Matn1+/+) mouse cartilage than that of MATN1−/− mice in vivo. However, such difference was not observed between Matn1+/+ and MATN1−/− chondrocytes cultured under stationary conditions in vitro. Cyclic loading significantly stimulated Acan and Col2a transcript levels in Matn1+/+ but not in MATN1−/− chondrocytes. This suggests that, while Matn1+/+ chondrocytes increase their anabolic gene expression in response to mechanical loading, the MATN1−/− chondrocytes fail to do so because of the deficiency in mechanotransduction. We also found that altered elastic modulus of cartilage matrix in Matn1−/− mice, suggesting the mechanotransduction has changed due to the deficiency of Matn1. To understand the impact of such deficiency on joint disease, mechanical loading was altered in vivo by destabilization of medial meniscus. While Matn1+/+ mice exhibited superficial fissures and clefts consistent with mechanical damage to the articular joint, Matn1−/− mice presented more severe cartilage lesions characterized by proteoglycan loss and disorganization of cells and ECM. This suggests that Matn1 deficiency affects pathogenesis of post-traumatic osteoarthritis by failing to up-regulate anabolic gene expression. This is the first demonstration of Matn1 function in vivo, which suggests its protective role in cartilage degeneration under altered mechanical environment.

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Introduction

Matrilin-1 (Matn1) is a cartilage specific non-collagenous extracellular matrix (ECM) protein [1], which has been implicated in diseases such as adolescent idiopathic scoliosis [2] and relapsing polychondritis [3]. Matn1 is a member of the matrilin family, which consists of four proteins sharing similar structural motifs such as von Willebrand factor A (vWFA) domains, epidermal growth factor (EGF)-like domains, and coiled-coil domains [4–6]. Matrilins form homo- or hetero-oligomers through assembly of C-terminal coiled-coil structures [5] and are known to have a bridging role in the ECM by connecting matrix components to form macromolecular networks. Matn1 interacts with type II collagen by coating the surface of collagen fibrils [7]. It also interacts with aggrecan, and such interaction is stabilized by cross-linking [8]. It has also been shown to associate with collagens type VI and IX and biglycan, thereby connecting these molecules to the aggrecan and type II collagen networks [9,10]. Indeed, a major phenotype of Matn1−/− mice is alteration of type II collagen fibrillogenesis and fibril organization [11]. The collagen fibril diameters are significantly increased, which leads to dense collagenous matrix structure in cartilage [2,11]. However, since skeletal development and growth plate morphology is apparently normal in MATN1−/− mice [11], the function of Matn1 in vivo in unclear.

Matn1 has also been proposed to play a role in mechanotransduction, since it is abundantly distributed in the pericellular matrix that is involved in transduction of mechanical signals to chondrocytes [12]. Mechanical transduction is the process of translating mechanical stimulation into cellular responses. Mechanical stress plays a fundamental role in regulating cellular activities during tissue morphogenesis and homeostasis [13–15]. Previous studies have shown that moderate cyclic loading stimulates chondrocyte functions by increasing the expression of anabolic chondrogenic ECM molecules such as type II collagen and aggrecan [10,12,13,16]. Elimination of matrilin content abolishes mechanical stimulation of chondrocyte proliferation and differentiation, and excessive or reduced Matn1 content decreases the mechanical response of chondrocytes [12]. It is proposed that pericellular Matn1 may affect overall mechanical adaptation of cartilage by regulating mechanical transduction of chondrocytes [12].

Given the function of Matn1 in regulating collagen fibril organization and in chondrocyte adaptability to mechanical loading, we propose the hypothesis that Matn1 deficiency would alter cartilage matrix mechanical property and chondrocyte mechanotransduction in response to mechanical loading. If this hypothesis is true, one would predict that Matn1 deficiency affects pathogenesis of post-traumatic osteoarthritis (PTOA), which is induced by altered mechanical environment in the joint [17–20]. In this study, we tested this hypothesis by characterizing articular cartilage in Matn1 wild-type (Matn1+/+), heterozygous (Matn1+/−) and knock-out (Matn1−/−) mice molecularly, mechanically and histologically.

Materials and Methods

RT-PCR expression analysis

Total RNA was isolated from rib, femoral head, and knee joint cartilage of 1 week old Matn1+/+ and Matn1−/− mice of both genders and from femoral head cartilage of 3 week-old Matn1+/+, MATN1+/+ and Matn1−/− mice of both genders using the RNAqueous Kit (Ambion, Austin, TX, USA) according to the manufacturer’s instructions. Gene expression analysis was conducted by real time quantitative PCR (RT-qPCR) with the DNA Engine Opticon 2 (Bio-Rad, Hercules, CA, USA) using the QuantiTect SYBR Green PCR kit (Qiagen). For RT-qPCR, 0.5 ug of RNA was reverse-transcribed using iScript™ cDNA synthesis kit (Bio-Rad) according to the manufacturer’s instructions. The cDNA of each sample was subjected to RT-qPCR using species-
specific primer pairs for genes encoding Matn1, type II collagen (Col2a1), aggrecan (Acan), Indian hedgehog (Ihh), type X collagen (Col X), runt-related transcription factor 2 (Runx2), matrix metalloproteinases 13 (MMP13) and a disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS5). Relative transcript levels were calculated using the delta-delta Ct (ΔΔCt) method, normalized to rRNA 18S expression according to the following equation: \( x = 2^{-\Delta\Delta Ct} \), in which \( \Delta\Delta Ct = \Delta E - \Delta C \), and \( \Delta E = Ct_{\text{exp}} - Ct_{18S}; \Delta C = Ct_{ctrl} - Ct_{18S} \). Ct_{ctrl} = Ct of control group.

**Mechanical testing of articular cartilage**

Atomic force microscopy (AFM) analysis was used to assess the mechanical changes in Matn1^{+/+} (n = 6), Matn1^{+/−} (n = 4), and Matn1^{−/−} (n = 5) articular cartilage surface. The femoral heads of 3-week-old mice of both genders were harvested for evaluation purposes. All freshly harvested samples were wrapped in phosphate-buffered saline (PBS)-soaked gauze and stored at 4°C for up to 2 days post-harvest, following previously established procedures [21]. Prior to testing, whole femurs were glued to a low profile 50 mm Petri dish to allow unobstructed access to the femoral head, and submerged in PBS to prevent drying of the tissue. Elastic moduli were quantitatively evaluated using an MFP-3D AFM (Asylum Research, Santa Barbara, CA) as described previously [13]. Briefly, spherically tipped, 5 μm diameter AFM cantilevers (k ~ 7.5 N/m, Novascan Technologies, Inc., Ames, IA) were used for elastic indentation tests. Indentation curves (10 μm/s approach velocity) were sampled at 5 kHz, with a force trigger between 50–300 nN prescribing the point at which the cantilever approach was stopped and then retracted (approximately 0.5–1 μm of indentation). For each testing region, 100 data points were collected, resulting in n = 400–600 indentations for each genotype group included in the study. Indentation curves were fit using a modified Hertz model to extract an elastic modulus for the cartilage surface [21].

**Mechanical response of chondrocytes**

Mouse primary chondrocytes were isolated from the rib cage cartilage of 1 week-old Matn1^{+/+} and Matn1^{−/−} mice of both genders. Cartilage slices were digested enzymatically in 0.2% collagenase type II. Individual cells were grown in Dulbecco’s Modified Eagle Medium (DMEM) containing 10% fetal calf serum. Then, Col2a1 and Acan expression were determined via real time RT-PCR.

Mechanical response of chondrocytes was determined in an established 3D chondrocyte culture system [22]. Chondrocytes cultured in 3D collagen scaffoldings form cartilage-like nodules that express specific markers of cartilage, including Col2a1 and Acan. After incubation of chondrocytes in collagen sponges overnight, the sponges were mechanically loaded to induce 5% elongation at 60 cycles/min, 15 min/h by a computer-controlled device (Bio-Stretch; ICCT Technologies, Markham, ON, Canada) for 48 hours. Bio-Stretch exerts a uniaxial stretch with square wave patterns, which induces extension of the collagen sponges at the x-axis and compression at the y-axis. At the indicated mechanical loading duration, chondrocytes were freed from sponges by collagenase digestion and collected for further analysis.

**Microarray analysis**

Rib cage cartilages were isolated from 1 week-old Matn1^{+/+} or Matn1^{−/−} mice of both genders. Rib cages were treated with 3mg/ml collagenase D (sigma) in DMEM medium supplemented with 2 mM L-glutamine, 0.05 mg/mL penicillin and 0.05 mg/mL streptomycin. After the first 90min treatment with collagenase D, rib cages were washed with Hank’s Balance Salt Solution (HBSS) by pipetting up and down to get rid of soft tissues surrounding ribs. After another 4
hours treatment with collagenase D or until the cartilages were completely digested, ribs were washed with HBSS solution. Chondrocytes in the supernatant were spun down and total RNA was isolated from them using the RNAqueous® Kit (Ambion, Austin, TX, USA) according to the manufacturer’s instructions. Total RNA samples were processed for microarray hybridization by the Genomics Core Facility at the Brown University Center for Genomics and Proteomics with GeneChip® Mouse Gene 1.0 ST Arrays (Affymetrix, USA). Biological triplicates were used for each genetic group. RNA gel electrophoresis is performed to examine RNA integrity. Global gene expression pattern with Principal Components Analysis mapping was generated with Partek Genomics suite 6.6 beta (Partek Incorporated, Missouri, USA).

Destabilization of the Medial Meniscus

After obtaining Rhode Island Hospital Institutional Animal Care and Use Committee approval, we performed a minimally invasive well known procedure called destabilization of the medial meniscus (DMM) to induce arthritis [23]. The procedure was performed on the right knees of Matn1⁻/⁻ (n = 11) and Matn1⁺/+ (n = 9) 2 month-old male mice. By transecting the meniscotibial ligament, a resultant displacement of the medial meniscus produced a mechanical instability of the ipsilateral knee. As a result, weight-bearing was focused in a smaller area on the medial aspects of the posterior femur and the central tibia. With unrestricted movement of the joint, an accelerated age-onset osteoarthritis (OA) model was induced by high focal mechanical stresses. Sham surgery was performed in which the ligament was visualized but not transected. Twenty-five mg/kg cefotaxime was injected after surgery to prevent infection, and 0.03mg/kg buprenorphine was injected for 2 days for pain relief. Following recovery, the mice were given unrestricted freedom to move for 8 weeks. Mice were then euthanized using CO₂ and the (experimental as well as control) knees were dissected and prepared for histological analysis.

Histology and scoring

Hind limbs dissected from Matn1⁻/⁻ and Matn1⁺/+ mice at 8 weeks post-operative were fixed overnight in 4% paraformaldehyde in phosphate-buffered saline (pH 7.4), decalcified in 0.2M EDTA for 3 weeks, dehydrated in 70%, 95%, and 99.9% ethanol, cleared in xylene, and embedded in paraaffin, and 6 μm sections were cut. Four sagittal sections were taken through the medial aspect of each specimen such that the articular surfaces of the tibia and femur were framed in the image. For Safranin-O/Fast Green staining, 5-μm paraffin-embedded sections of tibia from mice were counterstained with hematoxylin before being stained with 0.02% aqueous Fast Green for 4 minutes (followed by 3 dips in 1% acetic acid) and then 0.1% Safranin-O for 6 minutes. The slides were then dehydrated and mounted with crystal mount medium.

Eight sagittal sections were cut through the medial side of each specimen. All 8 sections from each specimen were considered separately and blindly by 2 investigators. Comparison of the Matn1⁻/⁻ and Matn1⁺/+ cartilage degradation was determined using a scoring system for the mouse knee developed by the Osteoarthritis Research Society International (OARSI) [24]. The femur and tibial articular surfaces on each section were scored on the 6-point OARSI scale. Sections in which there was no clear medial meniscus to help identify anatomic landmarks were discarded, leaving each specimen with 3 to 8 sections in which femur and tibial surfaces were clearly visualized. The OA scores for a given tibia and femur per specimen were defined as the maximum score for any of the sections. The maximum scores generated for each specimen were averaged together, producing four data sets: Matn1⁺/+ femur scores, Matn1⁺/+ tibial scores, Matn1⁻/⁻ femur scores, and Matn1⁻/⁻ tibial scores.
Statistical analysis

Histology grading was analyzed using a Kruskal-Wallis test with Dunn’s post-analysis. Gene expression and mechanical testing was done using two-way analysis of variance (ANOVA) followed by t-test analysis. Statistical significance was accepted at P < 0.05 for all analyses.

Results

Gene expression analysis

To determine whether gene expression of articular cartilage was altered in Matn1−/− mice, the mRNA levels of anabolic, catabolic, and hypertrophic markers were quantified via real time RT-PCR. Results confirmed the lack of Matn1 mRNA transcripts in the femoral head cartilage of Matn1−/− mice and reduced Matn1 transcript levels in Matn1+/− mice, relative to Matn1+/+ animals (Fig 1, i). Expression of anabolic genes, including cartilage ECM components (type II collagen and aggrecan), was significantly lower in the Matn1−/− and Matn1+/− mice compared with Matn1+/+ mice (Fig 1, ii and iii). Matn1−/− animals exhibited 50% less Acan and 80% less Col2a1 mRNA transcripts relative to Matn1+/+ animals. However, neither hypertrophic markers (Col X and Runx2) nor catabolic genes (MMP13 and ADAMTS5) showed a statistically significant difference among Matn1−/−, Matn1+/− and Matn1+/+ mice (Fig 1, v-vii). Thus, anabolic transcripts (Acan and Col2a1) levels were significantly reduced in Matn1 deficient cartilage.

Mechanical testing of articular cartilage

To determine whether the mechanical properties of cartilage tissue were altered in Matn1−/− mice, the elastic modulus of the surface of femoral head articular cartilage was quantified by atomic force microscopy (AFM). An average of 500 data points were collected from arrays of test sites at multiple locations areas on each specimen. The elastic modulus of Matn1−/− mice was significantly higher than that of Matn1+/+ and Matn1+/+ mice, with Matn1−/− cartilage exhibiting an elastic modulus (300 ± 70 kPa) double that of Matn1+/+ cartilage (150 ± 60 kPa) (Fig 2). Therefore, the mechanical property of cartilage matrix was altered in Matn1−/− mice in comparison to Matn1+/+ mice.

Transcriptome analysis of chondrocytes

To determine whether the reduction of anabolic transcripts in Matn1 deficient cartilage is consistent at different sites and ages, we quantified Col2a1 and Acan mRNA levels in femoral head and rib cartilage from one week old mice. There is a significant reduction of Col2a1 and Acan mRNA levels in both femoral head and rib cartilage (S1 Fig and Fig 3A). The reduction of anabolic transcripts (Acan and Col2a1) levels in Matn1 deficient cartilage could be due to an internal transcription deficiency in the nucleus of Matn1−/− chondrocytes. On the other hand, since Matn1 is a peri-cellular and ECM protein, the lack of it may affect chondrocyte transcription through cell signaling from the extracellular environment. To distinguish these two possibilities, we isolated chondrocytes from mouse cartilage by digesting ECM and plated the cells in monolayer without external mechanical loading. Real-time PT-PCR analysis demonstrated that, although the anabolic transcripts (Acan and Col2a1) levels were reduced in Matn1 deficient cartilage (Fig 3A), there was no significant difference in Col2a1 and Acan mRNA levels between Matn1+/+ and Matn1−/− chondrocytes isolated from cartilage (Fig 3B). This indicates that there is no internal deficiency of transcription of these anabolic transcripts in Matn1−/− chondrocytes in comparison to Matn1+/+ chondrocytes. To further determine whether there is significant difference in the overall expression of transcriptome between Matn1+/+ and Matn1−/− chondrocytes, microarray analysis of 28,206 probe sets (gene transcripts) was
performed using mRNA isolated from Matn1+/+ and Matn1−/− chondrocytes in monolayer culture. Principal Components Analysis indicated that there was no significant difference in the overall gene expression profiles between Matn1+/+ and Matn1−/− chondrocytes. Therefore, the basal levels of gene transcription, except Matn1, were similar between Matn1+/+ and Matn1−/− chondrocytes.

**Mechanical response of chondrocytes**

To test the possibility that the lack of Matn1 in ECM may affect chondrocyte transcription through mechanical signaling from the extracellular environment, we cultured Matn1−/− and
Matn1+/+ chondrocytes in a three dimensional collagen sponge subjected to cyclic loading of matrix scaffold (1 Hz, 5% matrix deformation, 15 min/hr). The levels of anabolic transcripts including Col2a1 and Acan mRNAs were quantified using real-time RT-PCR. In response to cyclic loading, Col2a1 and Acan mRNA expression levels were significantly increased in Matn1+/+ mouse chondrocytes (Fig 4, Matn1+/+). In contrast, mechanical stimulation of Col2a1 mRNA was abolished in Matn1−/− chondrocytes (Fig 4A, Matn1−/−). In addition, mechanical stimulation of Acan mRNA levels was significantly decreased in Matn1−/− chondrocytes in comparison to Matn1+/+ chondrocytes (Fig 4B, Matn1−/−). Furthermore, the difference of anabolic transcripts expression levels between Matn1+/+ and Matn1−/− chondrocytes only exist under mechanical loading (Fig 4, compare Load samples between Matn1+/+ and Matn1−/−), and there is no statistically significant difference of these transcripts under non-load conditions (Fig 4, compare Non-Load samples between Matn1+/+ and Matn1−/−). Thus, cyclic loading induced anabolic transcripts in Matn1+/+ chondrocytes and such induction was abolished in Matn1−/− chondrocytes.

Cartilage degeneration in response to altered mechanical environment
Deficiency of mechanical activation of anabolic chondrocytes may affect cartilage degeneration in response to altered mechanical loading environment. To test this hypothesis, we altered
Fig 3. Comparison of gene expression levels in A) cartilage of Matn1\textsuperscript{−/−} and Matn1\textsuperscript{+/+} mice, B) primary chondrocytes of Matn1\textsuperscript{−/−} and Matn1\textsuperscript{+/+} mice cultured under monolayer cell culture conditions. Total RNA was isolated from A) rib cartilage and B) cultured primary chondrocytes of rib cartilage of 1 week old Matn1\textsuperscript{+/+} and Matn1\textsuperscript{−/−} mice. Gene expression analysis was conducted by real time quantitative PCR (RT-qPCR). The cDNA of each sample was subjected to RT-qPCR using for anabolic genes encoding i) type II collagen (Col2a1); ii) aggrecan (Acan). Values are the mean ± SD. *p<0.05. (n = 3)

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mechanical loading of articular cartilage by destabilization of medial meniscus (DMM) in the mouse knee. In mouse knee articular cartilage, the mRNA levels of Acan and Col2a1 were significantly reduced in Matn1−/− mice (Fig 5A), similar to rib and femoral head cartilage. Histology analysis was performed following DMM procedure. Histological scoring indicated that the Matn1−/− femur cartilage sustained a greater degree of erosion than the Matn1+/+ cartilage. The results showed that the average maximum OARSI score for Matn1−/− femoral cartilage was 3.9 ± 1.5, significantly higher than Matn1+/+ (1.9 ± 1.5) (p < 0.05), (Fig 5B, Femur). On the other hand, the Matn1+/+ tibial cartilage was more severely damaged by DMM procedure than femoral cartilage and there was no statistically significant increase of the OARSI score in Matn1−/− tibial cartilage in comparison to Matn1+/+ (Fig 5B, Tibia).

In addition, the overall morphology of cartilage erosion patterns differed between Matn1−/− and Matn1+/+ specimens (Fig 5C). While fissures and clefts of the superficial layer were more commonly seen in Matn1+/+ cartilage specimens (Fig 5C i), irregular and massive degenerative matrix changes predominated in Matn1−/− specimens (Fig 5C ii). Loss of proteoglycan is evident in articular cartilage of the Matn1−/− specimens, as indicated by the severe reduction of Safranin O staining in the matrix. Furthermore, the cartilage zones and surrounding matrix are disorganized, suggesting deficiency in ECM organization in Matn1−/− articular cartilage.

Discussion and Conclusions

The function of MATN1 in vivo was not known, since cartilage development was normal in Matn1−/− mice [11]. However, previous studies have shown that the diameters of collagen fibrils in cartilage were increased [2,11] suggesting the content and/or mechanical properties of ECM in Matn1−/− mouse cartilage may be altered. We hypothesize that Matn1 may play a role in protecting cartilage from degeneration and such a role may only manifest when cartilage is tested under altered mechanical environment. To test this hypothesis in vivo, we performed destabilization of medial meniscus (DMM) procedure in Matn1−/− mice. This approach was utilized because Matn1 is expressed specifically in mature chondrocytes [1]. The cartilage tissue and
differentiation stage specific expression of Matn1 ensures that any phenotypic changes are due to chondrocyte autonomous effects in cartilage, but not from effects of other tissues in vivo.

The second important consideration is that the morphology of growth plate and articular cartilage is apparently normal in the Matn1−/− mice [11]. This eliminates the alteration of cartilage morphology as a confounding variable for studying cartilage’s response to mechanical loading. Third, we have shown previously that the content of pericellular Matn1 is critical to optimal chondrocyte mechanotransduction in vitro [12]. We tested whether this conclusion also holds true in vivo in this study.

Alteration of mechanical loading to cartilage joint is performed by DMM procedure. It is selected for its relatively mild effect of mechanical damage on cartilage matrix and for its minimal activation of pro-inflammatory pathways in the joint [23]. Histology analysis indicates that, following DMM procedure, instead of typical fissures and clefts commonly seen in articular cartilage surface of Matn1+/+ mice, irregular and massive degenerative erosion predominated in Matn1−/− mice. Notably, proteoglycan accumulation is diminished and zonal organization of chondrocytes is lacking in Matn1−/− cartilage. This strongly suggests that the mechanism underlying degenerative changes in Matn1−/− mice is more severe than that of the Matn1+/+ mice. Interestingly, such histological changes are only seen in Matn1−/− mice following DMM but not in those mice without DMM. Thus, altered mechanical environment triggers
manifestation of the new cartilage degenerative phenotype in Matn1−/− mice. These results demonstrated, for the first time, the importance of MATN1 in cartilage homeostasis and its preventive role in OA progression. Such properties have only been revealed in Matn1 deficient mice under post-traumatic conditions.

Histology analysis suggests that Matn1 deficiency may alter the content of ECM in cartilage. Such alteration could be due to the reduction of synthesis and/or increased degradation of ECM. To test this, we quantified expression of key homeostatic genes expressed by chondrocytes. First, we analyzed the anabolic genes of major ECM components in cartilage, specifically, Acan and Col2a1. Both genes were down-regulated in Matn1−/− mice. Chondrocytes from Matn1−/− mice also showed a trend of lower expression of both genes indicating that the production of ECM was suppressed in Matn1−/− mice. This is the first evidence to show that ECM production is affected by Matn1 deficiency in mammals. In support of this conclusion, a recent study has shown that collagen production and secretion is reduced when Matn1 is knocked down in zebra fish [25]. We also quantified mRNA levels of Col6a1 and Decorin, which interact with Matn1. However, they were not significantly different between the Matn1+/+ and Matn1−/− mice (data not shown). Therefore, the effect of Matn1 on matrix production may be gene specific.

Because cartilage degeneration can be caused not only by the decrease of anabolic gene expression, but also by activation of chondrocyte hypertrophy and increase of catabolic gene expression [26], we quantified the expression of hypertrophic markers and catabolic genes in cartilage. Neither the expression levels of hypertrophy markers Col X and Runx2 nor those of catabolic genes MMP13 and ADAMTS5 were significantly different among Matn1−/−, Matn1−/− and Matn1−/− mice. These findings strongly suggest that decrease of ECM production, but not stimulation of chondrocyte hypertrophy or matrix degradation was responsible for the more severe cartilage lesions observed in Matn1−/− mice during PTOA.

Changes of ECM production may result in alteration of material properties of cartilage matrix. To test whether the mechanical property of Matn1 deficient cartilage was altered, we quantified the elastic modulus of mouse articular cartilage surface by AFM. Surprisingly, the elastic modulus of Matn1−/− mice was more than double that of Matn1−/− mice, indicating a much stiffer matrix in Matn1−/− cartilage. There are at least two explanations as to why the stiffness is increased in Matn1−/− cartilage. First, due to the lack of Matn1 on the surface of collagen fibrils, the collagen fibrils aggregate to form dense collagenous structure [9–11]. Since type II collagen has a long half-life, the accumulated, dense collagen fibrils contribute to the increase of the elastic modulus despite the decrease of new collagen production in Matn1−/− cartilage. On the other hand, the half-life of aggrecan is much shorter than that of collagens in the matrix. Since the decrease of proteoglycan content has been shown to increase cartilage stiffness [27], the decrease of proteoglycan production would significantly impact cartilage mechanical properties in Matn1 deficient mice.

Chondrocyte mechanotransduction could be affected by altered matrix property as well as by the lack of peri-cellular Matn1, which has previously been shown to be responsible for transducing matrix deformation signals to chondrocytes in vitro [12]. To test cellular response to mechanical loading, we cultured Matn1+/+ and Matn1−/− mouse chondrocytes in 3D collagen scaffolding subjected to cyclic loading. In response to mechanical loading, the mRNA levels of Col2a1 and Acan were significantly increased in Matn1−/− chondrocytes. However, this mechanical stimulation is diminished in Matn1−/− chondrocytes. Thus, Matn1−/− chondrocytes are deficient in stimulating ECM production in response to mechanical strain. Interestingly, our results suggested that the synthetic defect of Matn1−/− chondrocytes is not the cause of the ECM production deficiency. Matn1−/− chondrocytes have the same Col2a1 and Acan mRNA levels as the Matn1+/+ chondrocytes when cultured in monolayer and in 3D cultures without
external loading. Furthermore, there is no significant difference in the overall expression profiles of transcriptome between \textit{Matn1}^{+/+} and \textit{Matn1}^{-/-} chondrocytes, as indicated by microarray analysis. This suggested that a major defect of \textit{Matn1}^{-/-} chondrocytes is the lack of sensitivity to mechanical environment, rather than any deficiency in mRNA synthesis. This enables \textit{Matn1}^{-/-} mice to be an ideal model for testing the role of mechanotransduction \textit{in vivo}.

Although chondrocytes residing in mouse articular cartilage show a significant difference in anabolic gene expression (i.e., \textit{Acan} and \textit{Col2a1}) between \textit{Matn1}^{+/+} and \textit{Matn1}^{-/-} mice \textit{in vivo}, such difference is lost when the same chondrocytes are isolated from the surrounding ECM and cultured \textit{in vitro}. This difference in anabolic gene expression, however, can be restored by applying external cyclic loading \textit{in vitro}. These observations suggest that articular cartilage is a mechanically active tissue \textit{in vivo}, and that matrix molecules such as Matn1 is essential for chondrocytes to adapt to mechanical environment. Thus, when mechanical loading environment is altered, such as during PTOA, chondrocytes in \textit{Matn1}^{+/+} mice are able to adapt to mechanical stress by increasing the synthesis of ECM molecules such as Col II, Acan, and matrilins [28,29], while chondrocytes in Matn1 deficient mice fail to adapt to mechanical stress. This failure results in a diminished ability of chondrocytes to maintain cartilage homeostasis, which eventually leads to more severe cartilage lesions during PTOA. One limitation of the study is that the mRNA levels by RT-PCR analysis have not been confirmed by protein analysis due to technical challenges. Such analysis at protein levels will be performed in future experiments.

In summary, our working model (Fig 6) is that mechanotransduction plays an important role for chondrocytes to adapt to altered mechanical environment during PTOA. This important role is revealed by the analysis of \textit{Matn1}^{-/-} mice presented here. The lack of Matn1 results in aggregation of collagen bundles and decrease of aggrecan content. These changes lead to stiffer matrix mechanical property, which, together with the absence of pericellular Matn1, diminish chondrocyte mechanotransduction ability. Our data suggests that, in addition to the commonly recognized stimulation of catabolic genes, the failure of stimulation of anabolic genes by mechanical loading also contributes to pathogenesis of OA.

**Supporting Information**

S1 Fig. Gene expression levels of young articular cartilage from \textit{Matn1}^{-/-} and \textit{Matn1}^{+/+} mice. Total RNA was isolated from femoral head of 1 week-old \textit{Matn1}^{+/+}, \textit{Matn1}^{-/-} mice. The
cDNA of each sample was subjected to RT-qPCR using species-specific primer pairs for ana-
bolic genes encoding aggrecan (Acan) and type II collagen (Col2a1). Values are the mean ±
SD. *p<0.05 compared to Matn1+/+ (n≥3).

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**Author Contributions**

Conceived and designed the experiments: YC QC. Performed the experiments: YC JC CJ XL
YG VF KY CC HY. Analyzed the data: YC JC KK PM ED QC. Contributed reagents/materials/
analysis tools: KK PM ED QC. Wrote the paper: YC JC QC.

**References**

1. Chen Q., Johnson D. M., Haudenschild D. R., and Goetinck P. F. (1995) Progression and recapitulation
   of the chondrocyte differentiation program: cartilage matrix protein is a marker for cartilage maturation.
   Dev Biol 172, 293–306 PMID: 7589809

2. Nicolae C., Ko Y. P., Miosge N., Niehoff A., Studer D., Enggist L., et al. (2007) Abnormal collagen fibrils
   in cartilage of matrilin-1/matrilin-3-deficient mice. J Biol Chem 282, 22163–22175 PMID: 17502381

3. Chen Z., Tang N. L., Cao X., Qiao D., Yi L., Cheng J. C., et al. (2009) Promoter polymorphism of matri-
   lin-1 gene predisposes to adolescent idiopathic scoliosis in a Chinese population. Eur J Hum Genet
   17, 525–532 doi: 10.1038/ejhg.2008.203 PMID: 18985072

4. Deak F., Wagener R., Kiss I., and Paulsson M. (1999) The matrilins: a novel family of oligomeric extra-
cellular matrix proteins. Matrix Biol 18, 55–64 PMID: 10367731

5. Klatt A. R., Nitsche D. P., Kobbe B., Morgelin M., Paulsson M., and Wagener R. (2000) Molecular struc-
ture and tissue distribution of matrilin-3, a filament-forming extracellular matrix protein expressed during
skeletal development. J Biol Chem 275, 3999–4006 PMID: 10660556

6. Paulsson M., and Heinegard D. (1982) Radioimmunoassay of the 148-kilodalton cartilage protein. Dis-
tribution of the protein among bovine tissues. Biochemical Journal 207, 207–213 PMID: 7193980

7. Winterbottom N., Tondravi M. M., Harrington T. L., Klier F. G., Vertel B. M., and Goetinck P. F. (1992)
Cartilage matrix protein is a component of the collagen fibril of cartilage. Dev Dyn 193, 266–276 PMID:
1600245

8. Hauser N., Paulsson M., Heinegard D., and Morgelin M. (1996) Interaction of cartilage matrix protein
with aggrecan. Increased covalent cross-linking with tissue maturation. J Biol Chem 271, 32247–
32252 PMID: 8943283

9. Fresquet M., Jowitt T. A., Stephen L. A., Ylostalo J., and Briggs M. D. (2010) Structural and functional
investigations of Matrilin-1 A-domains reveal insights into their role in cartilage ECM assembly. J Biol
Chem 285, 34048–34061 doi: 10.1074/jbc.M110.154443 PMID: 20729554

10. Wiberg C., Klatt A. R., Wagener R., Paulsson M., Bateman J. F., Heinegard D., et al. (2003) Complexes
of matrilin-1 and biglycan or decorin connect collagen VI microfibrils to both collagen II and aggrecan. J
Biol Chem 278, 37698–37704 PMID: 12940020

11. Huang X., Birk D. E., and Goetinck P. F. (1999) Mice lacking matrilin-1 (cartilage matrix protein) have
alterations in type II collagen fibrillogenesis and fibril organization. Developmental Dynamics 216, 434–441
PMID: 10633862

12. Kanbe K., Yang X., Wei L., Sun C., and Chen Q. (2007) Pericellular matrilins regulate activation of
chondrocytes by cyclic load-induced matrix deformation. J Bone Miner Res 22, 318–328 PMID:
17129169

13. Darling E. M., Wilusz R. E., Bolognesi M. P., Zauscher S., and Guilak F. (2010) Spatial mapping of the
biomechanical properties of the pericellular matrix of articular cartilage measured in situ via atomic
force microscopy. Biophys J 98, 2848–2856 doi: 10.1016/j.bpj.2010.03.037 PMID: 20550897
14. Fujisawa T., Hattori T., Takahashi K., Kuboki T., Yamashita A., and Takigawa M. (1999) Cyclic mechanical stress induces extracellular matrix degradation in cultured chondrocytes via gene expression of matrix metalloproteinases and interleukin-1. J Biochem 125, 966–975 PMID:10220591

15. Li Z., Kupcsik L., Yao S. J., Alini M., and Stoddart M. J. (2010) Mechanical load modulates chondrogenesis of human mesenchymal stem cells through the TGF-beta pathway. J Cell Mol Med 14, 1338–1346 doi: 10.1111/j.1582-4934.2009.00780.x PMID: 19432813

16. Guan Y. J., Yang X., Wei L., and Chen Q. (2011) MiR-365: a mechanosensitive microRNA stimulates chondrocyte differentiation through targeting histone deacetylase 4. FASEB J 25, 4457–4466 doi: 10.1007/s00167-014-2932-6 PMID: 24633008

17. Fleming B. C., Proffen B. L., Vavken P., Shalvoy M. R., Machan J. T., and Murray M. M. (2015) Increased platelet concentration does not improve functional graft healing in bio-enhanced ACL reconstruction. Knee Surg Sports Traumatol Arthrosc 23, 1161–1170 doi: 10.1007/s00167-014-2932-6 PMID: 24633008

18. Natoli R. M., and Athanasiou K. A. (2009) Traumatic loading of articular cartilage: Mechanical and biological responses and post-injury treatment. Biochemistry 46, 451–485 doi: 10.3333/BIR-2009-0554 PMID: 20164631

19. Nordenvall R., Bahmanyar S., Adami J., Mattila V. M., and Fellander-Tsai L. (2014) Cruciate ligament reconstruction and risk of knee osteoarthritis: the association between cruciate ligament injury and post-traumatic osteoarthritis. a population based nationwide study in Sweden, 1987–2009. PLoS One 9, e104681 doi: 10.1371/journal.pone.0104681 PMID: 25148530

20. von Porat A., Roos E. M., and Roos H. (2004) High prevalence of osteoarthritis 14 years after an anterior cruciate ligament tear in male soccer players: a study of radiographic and patient relevant outcomes. Ann Rheum Dis 63, 269–273 PMID: 14962961

21. Coles J. M., Blum J. J., Jay G. D., Darling E. M., Guilak F., and Zauscher S. (2008) In situ friction measurement on murine cartilage by atomic force microscopy. J Biomech 41, 541–548 PMID: 18054362

22. Yang X, Vezeridis PS, Nicholas B, Crisco JJ, Moore DC, Chen Q. Differential expression of type X collagen in a mechanically active 3-D chondrocyte culture system: a quantitative study. J Orthop Surg Res. 2006 Dec 6; 1:15. PMID:17150098

23. Glasson S. S., Blanchet T. J., and Morris E. A. (2007) The surgical destabilization of the medial meniscus (DMM) model of osteoarthritis in the 129/SvEv mouse. Osteoarthritis Cartilage 15, 1061–1069 PMID: 17470400

24. Glasson S. S., Chambers M. G., Van Den Berg W. B., and Little C. B. (2010) The OARSI histopathology initiative—recommendations for histological assessments of osteoarthritis in the mouse. Osteoarthritis Cartilage 18 Suppl 3, S17–23 doi: 10.1016/j.joca.2010.05.025 PMID: 20864019

25. Neacsu C. D., Ko Y. P., Tagariello A., Rokenes Karlsen K., Neiss W. F., Paulsson M., et al. (2014) Matrilin-1 is essential for zebrafish development by facilitating collagen II secretion. J Biol Chem 289, 1505–1518 doi: 10.1074/jbc.M113.529933 PMID: 24293366

26. van der Kraan P. M., and van den Berg W. B. (2012) Chondrocyte hypertrophy and osteoarthritis: role in initiation and progression of cartilage degeneration? Osteoarthritis Cartilage 20, 223–232 doi: 10.1016/j.joca.2011.12.003 PMID: 22178514

27. Stolz M., Raiteri R., Daniels A. U., VanLandingham M. R., Baschong W., and Aebi U. (2004) Dynamic Elastic Modulus of Porcine Articular Cartilage Determined at Two Different Levels of Tissue Organization by Indentation-Type Atomic Force Microscopy. Biophysical Journal 86, 3269–3283 PMID: 15111440

28. Anderson D. D., Chubinskaya S., Guilak F., Martin J. A., Oegema T. R., Olson S. A., et al. (2011) Post-traumatic osteoarthritis: improved understanding and opportunities for early intervention. J Orthop Res 29, 802–809 doi: 10.1002/jor.21359 PMID: 21520254

29. Okimura A., Okada Y., Makihira S., Pan H., Yu L., Tanne K., et al. (1997) Enhancement of cartilage matrix protein synthesis in arthritic cartilage. Arthritis Rheum 40, 1029–1036 PMID: 9182912