The difference in joint instability affects the onset of cartilage degeneration or subchondral bone changes

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Objective: It has been debated whether the onset of knee osteoarthritis is initiated in cartilage or subchondral bone. The purpose of this study was to clarify the effects of increasing or decreasing joint instability on cartilage degeneration and subchondral bone changes in knee OA by comparing different models of joint instability.

Methods: We used the anterior cruciate ligament transection (ACL-T) model and the destabilization of the medial meniscus (DMM) model. In addition, we created a controlled abnormal tibial translation (CATT) and a controlled abnormal tibial rotation (CATR) model. We performed joint instability analysis, micro-computed tomography analysis, histological and immunohistological analysis in 4 and 6 weeks.

Results: The CATT group suppressed joint instability in the ACL-T group (6 weeks; $P = 0.032$), and the CATR group suppressed joint instability in the DMM group (6 weeks; $P = 0.032$). Chondrocyte hypertrophy in the ACL-T and DMM groups was increased compared to the Sham group (6 weeks; [ACL-T vs Sham], $P = 0.002, 95\% CI [5.983–33.025]$; [DMM vs Sham], $P = 0.022, 95\% CI [1.691–28.733]$). In the subchondral bone, the BV/TV in the DMM and CATR groups was increased compared to the ACL-T and CATT groups (6 weeks; [DMM vs ACL-T], $P = 0.002, 95\% CI [7.404–37.582]$; [DMM vs CATT], $P = 0.014, 95\% CI [2.881–33.059]$; [CATR vs ACL-T], $P = 0.006, 95\% CI [4.615–34.793]$; [CATR vs CATT], $P = 0.048, 95\% CI [0.092–30.270]$).

Conclusions: This study showed that joint instability promotes chondrocyte hypertrophy, but subchondral bone changes were influenced by differences in ACL and meniscus function.

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Introduction

Osteoarthritis (OA) is a degenerative joint disease characterized by articular cartilage degeneration, osteophyte formation, and subchondral bone sclerosis1–2. Knee OA has the highest morbidity among OA, and interferes with daily life due to pain and dysfunction of the knee joint. Radin et al., in 1986 proposed that the imbalance of subchondral bone stiffness may be the cause of articular cartilage damage. Since then, it has been debated whether the onset of knee OA is initiated in the cartilage or the subchondral bone2. Some reports have shown that articular cartilage degeneration precedes changes in subchondral bone4,6. However, other reports suggested that changes in subchondral bone precede articular cartilage degeneration6,7. Therefore, there was no consistent view on this topic yet.

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Abbreviations: ACL-T, Anterior Cruciate Ligament Transection; CATT, Controlled Abnormal Tibial translation; CATR, Controlled Abnormal Tibial Rotation; DMM, Destabilization of the Medial Meniscus; OA, Osteoarthritis; MMTL, Medial Meniscal Ligament; ICR, Institute for Cancer Research; µCT, Micro-computed tomography; BV/TV, Bone Volume/Tissue Volume; Tb.Th, Trabecular Thickness; Tb.N, Trabecular Number; Tb.Sp, Trabecular Separation; OARSI, Osteoarthritis Research Society International; TRAP, Tartrate-Resistant Acid Phosphatase; OCS/BS, Osteoclast Surface/Bone Surface; Col X, Type X collagen; CI, Confidence intervals.

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The mechanism of knee OA onset has not been elucidated, but mechanical stress is considered to be the main factor. Although excessive mechanical stress causes articular cartilage degeneration, moderate mechanical stress contributes to articular cartilage protection. In addition, a study that had used an animal model of knee OA revealed that moderate exercise with a treadmill suppressed the progression of knee OA, and, high-intensity exercise promoted the progression of knee OA. Our laboratory has reported that joint instability was regarded as abnormal mechanical stress and that suppression of joint instability delays cartilage degeneration.

Two popular rodent knee OA model includes the anterior cruciate ligament transection (ACL-T) model and the destabilization of the medial meniscus (DMM) model. The ACL-T model transects the ACL, and the DMM model transects the medial meniscotibial ligament (MMTL). These models cause OA by transecting the ligaments involved in knee joint stability. Therefore, these models both induce joint instability. It has been reported that articular cartilage degeneration depends on the magnitude of joint instability, and the DMM model has a slower progression of cartilage degeneration and less joint instability than the ACL-T model. However, the DMM model showed early degeneration of the meniscus, suggesting that not only joint instability but also dysfunction of the meniscus may be involved in the development of OA. Therefore, we applied the controlled abnormal tibial translation (CATT) model, which suppresses joint instability caused by the ACL-T model, to the DMM model. Thus, we established a new controlled abnormal tibial rotation (CATR) model to suppress joint instability in the DMM model, and can evaluate the process of knee OA development with suppressed joint instability in the DMM model. Furthermore, by comparing the new model with the existing ACL-T model, DMM model, and CATT model, we can examine the effects of joint instability and the differences in the effects of ACL and meniscus dysfunction on knee OA.

Therefore, the purpose of this study was to clarify the effects of increasing or decreasing in a different type of joint instability on cartilage degeneration and subchondral bone changes in knee OA. We compared the ACL-T model, CATT model, DMM model, and CATR model and clarified part of the knee OA development mechanism by comparing the models.

Materials and methods

Animals and experimental design

This study was approved by the Animal Research Committee of Saitama Prefectural University (approval number: 2020-1), and the animals were handled in accordance with the relevant legislation and institutional guidelines for humane animal treatment. In this study, 59 adult (12-week-old) Institute for Cancer Research (ICR) male mice were randomized into one of five groups: ACL-T group (ACL-T, n = 12), CATT group (CATT, n = 12), DMM group (DMM, n = 12), CATR group (CATR, n = 12), and sham group (Sham, n = 11). We sacrificed all mice at 4 and 6 weeks after surgical intervention (4 weeks; each group: n = 6, 6 weeks; Sham group: n = 5, other groups: n = 6). All mice were housed in plastic cages, and the room had a 12-hour light/dark cycle. Mice were permitted unrestricted movement within the cage and had free access to food and water.

Surgical procedures

All surgical procedures were performed on the left knee joint with the mice under a combination anesthetic (medetomidine, 0.375 mg/kg; midazolam, 2.0 mg/kg; and butorphanol, 2.5 mg/kg).
The medial capsule was exposed in the ACL-T group, and scissors were used to cut the ACL. CATT was performed following the same procedure as the ACL-T surgery [Fig. 1(A)]. Mice assigned to the CATT group had bone tunnels created in the distal femur and proximal tibia using a 25-gauge needle and 4-0 nylon threads threaded through them to suppress the anterior-posterior joint instability that occurs in the ACL-T model [Fig. 1(B)a,b]. Subsequently, 4-0 nylon threads were tightly tied [Fig. 1(B)c] to compensate for ACL function and suppress anterior-posterior joint instability.

DMM surgery was performed on the left knee joint of the mice as previously described13. CATR surgery was performed following the same procedure as DMM surgery. Mice assigned to the CATR group were created using the same procedure as the CATT model. The bone tunnels were positioned vertically to suppress rotational instability that occurs in the DMM model [Fig. 1(A)]. To eliminate differences between the groups, bone tunnels were created in the Sham, ACL-T, and DMM groups as well as in the CATT and CATR groups, and a nylon thread was tied loosely so as not to suppress joint instability.

Anterior drawer test
To assess anterior-posterior joint instability, we performed the anterior drawer test using a constant force spring (0.05 kgf; Sanko Spring Co., Ltd., Fukuoka, Japan) and a soft X-ray device (M-60; Softex Co., Ltd., Kanagawa, Japan)13. At 4 and 6 weeks after surgery, we collected the knee joints of mice with an intact femur, tibia, and paw. For the measurement of displacement, photographs were taken with the knee joint in 90° of flexion and the femur fixed to a clamp and with the tibia pulled forward using constant force spring. Digital images were acquired using an x-ray sensor (Naomi; RF Co. Ltd., Nagano, Japan) with a voltage of 28 kV, a current of 1.5 mA, and an exposure time of 1 s. The soft x-ray images were used to quantify the anterior displacement of the tibia using a dedicated image analysis software (ImageJ; National Institutes of Health, Bethesda, MD, USA). The anterior displacement was measured by the linear distance from the perpendicular line from the lower end of the femoral condyle to the posterior edge of the tibial joint surface.

Tibial rotational test
To assess rotational joint instability, we performed a tibial rotational test using a constant force spring (0.05 kgf; Sanko Spring Co., Ltd., Fukuoka, Japan) and a soft x-ray device (M-60; Softex Co., Ltd., Kanagawa, Japan)13. At 4 and 6 weeks after surgery, we collected the knee joints of mice with an intact femur, tibia, and paw. For the measurement of displacement, photographs were taken with the knee joint in 90° of flexion and the femur fixed to a clamp and with the tibia pulled forward using constant force spring. Digital images were acquired using an x-ray sensor (Naomi; RF Co. Ltd., Nagano, Japan) with a voltage of 28 kV, a current of 1.5 mA, and an exposure time of 1 s. The soft x-ray images were used to quantify the anterior displacement of the tibia using a dedicated image analysis software (ImageJ; National Institutes of Health, Bethesda, MD, USA). The anterior displacement was measured by the linear distance from the perpendicular line from the lower end of the femoral condyle to the posterior edge of the tibial joint surface.

Histological analysis
After evaluation of joint instability, the harvested knee joint was fixed with 4% paraformaldehyde for 1 day, decalcification in 10% ethylenediaminetetraacetic acid for 3 weeks, dehydrated and embedded in paraffin. The samples were cut in the sagittal plane (6 µm thickness) using a microtome (ROM-360; Yamato Kohki Industrial Co., Ltd., Saitama, Japan). Safranin-O/fast green staining was performed to evaluate the articular cartilage degeneration. The Osteoarthritis Research Society International (OARSI) histopathological grading system was used to assess cartilage degeneration20 by two independent observers (KT and SN) who were blinded to all other sample information. We used the medial tibial plateau for analysis. And, one section without meniscal coverage was used for analysis. The average value of the scores of the two observers was used as the representative value.

Immunohistochemical analysis and TRAP staining
To evaluate the expression of type X collagen (Col X) and Osterix, we performed immunohistochemical staining using the avidin-biotinylated enzyme complex method and the VECTASTAIN Elite ABC Rabbit IgG Kit (Vector Laboratories, Burlingame, CA, USA). The tissue sections were deparaffinized with xylene and ethanol, and antigen activation was performed using proteinase K (Worthington Biochemical Co., Lakewood, NJ, USA) for 15 min. Endogenous peroxidase was inactivated with 0.3% H2O2/methanol for 30 min. Nonspecific binding of the primary antibody was blocked using normal goat serum for 30 min. The sections were then incubated with anti-collagen X antibody (1:300, ab58632, Abcam) and Osterix polyclonal antibody (1:250, bs-1110R, Bioss) overnight at 4°C. Afterward, the sections were incubated with biotinylated secondary anti-rabbit IgG antibody and stained using Dako Liquid DAB + Substrate Chromogen System (Dako, Glostrup, Denmark).

Cell nuclei were stained with hematoxylin. For analysis, we calculated the ratio between the number of Col X positive cells and the number of chondrocytes in an articular cartilage area of 10,000 µm² (100 µm × 100 µm). In the analysis of Osterix, the positive cell ratios in the medullary cavity of the subchondral bone at 2,500 µm² (50 µm × 50 µm) were compared. We calculated Osterix positive cell ratio from the total number of bone marrow cells at two locations in the medullary cavity of 2,500 µm² (50 µm × 50 µm) and the total number of Osterix positive cells. According to manufacturer instructions, osteoclast activity was detected by histochemical staining for tartrate-resistant acid phosphatase (TRAP) using the TRAP stain kit (Wako, Osaka, Japan). We then calculated the osteoclast surface/bone surface (OC/S/BS, %) in the bone marrow region as an analysis of osteoclast activity.

Statistical analysis
All data were analyzed using R software, version 3.6.1. First, the normality of all data was verified using the Shapiro–Wilk test.
Since the evaluation of joint instability and the OARSI score were nonparametric, the Kruskal–Wallis test was used. Subsequently, the Steel–Dwass test was used for post-hoc analysis. A one-way analysis of variance (ANOVA) was performed for the other data, followed by a post hoc Tukey–Kramer test. Parametric data were expressed as means with 95% confidence intervals (CI), whereas non-parametric data were expressed as medians with interquartile ranges. The statistical significance was set at \( P < 0.05 \).

Results

Evaluation of joint instability

Anterior-posterior joint instability was quantified in terms of anterior displacement of the tibia using the anterior drawer test [Fig. 2(A)]. At 4 weeks, the anterior displacement of the ACL-T group increased significantly compared to that of the other groups. The anterior displacement of the CATT group significantly reduced the joint instability of the ACL-T group, but significantly increased it compared to the Sham, DMM, and CATR groups. ([ACL-T vs Sham], \( P = 0.031 \); [ACL-T vs CATT], \( P = 0.032 \); [ACL-T vs DMM], \( P = 0.031 \); [ACL-T vs CATR], \( P = 0.032 \); [CATT vs Sham], \( P = 0.032 \); [CATT vs DMM], \( P = 0.032 \); [CATT vs CATR], \( P = 0.032 \)).

At 6 weeks, the anterior displacement of the ACL-T group increased significantly compared to that of the other groups. On the other hand, the CATT group significantly reduced the anterior displacement of the ACL-T group, and no significant difference compared to the other groups ([ACL-T vs Sham], \( P = 0.047 \); [ACL-T vs CATT], \( P = 0.032 \); [ACL-T vs DMM], \( P = 0.032 \); [ACL-T vs CATR], \( P = 0.032 \)).

Rotational joint instability was quantified in terms of change in the tibial rotation angle using a tibial rotational test [Fig. 2(B)]. At 4 weeks, the change in tibial rotation angle in the DMM group was not significantly different from those in the Sham group but was significantly increased compared to that of the other groups. In contrast, the change in the tibial rotation angle in the CATR group was not significantly different from that in the Sham, ACL-T, and CATT groups ([DMM vs ACL-T], \( P = 0.032 \); [DMM vs CATT], \( P = 0.032 \); [DMM vs CATR], \( P = 0.032 \)).

Similarly, at 6 weeks, the change in tibial rotation angle in the DMM group was not significantly different from that in the Sham group but was significantly increased compared to that of the ACL-T and CATR groups. In contrast, the change in the tibial rotation angle in the CATR group was not significantly different from that in the Sham, ACL-T, and CATT groups ([DMM vs ACL-T], \( P = 0.032 \); [DMM vs CATT], \( P = 0.032 \)).

Subchondral bone changes between models

The 3D reconstruction images of the knee joint by \( \mu \)CT analysis are shown in Fig. 3(A). At 4 and 6 weeks, lateral displacement of the medial meniscus was observed in all samples in the DMM and CATR groups, but not in all other groups.

At 4 weeks, the BV/TV in the DMM group was significantly higher than that in the CATT group. (\( P = 0.043, 95\% \text{CI} [0.325–28.815] \)). Although there was no significant difference, the BV/TV in the ACL-T and CATT groups was lower than that in the Sham group, and the BV/TV in the DMM group was higher than that in the Sham group. 

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At 6 weeks, the BV/TV in the DMM and CATR groups was significantly higher than that in the ACL-T and CATT groups ([DMM vs ACL-T], \( P = 0.002, 95\%CI \ [7.404–37.582] \)); [DMM vs CATT], \( P = 0.014, 95\%CI \ [2.881–33.059] \)); [CATR vs ACL-T], \( P = 0.006, 95\%CI \ [4.615–34.793] \)); [CATR vs CATT], \( P = 0.048, 95\%CI \ [0.092–30.270] \)). Although there was no significant difference, the BV/TV in the ACL-T and CATT groups was lower than that in the Sham group, and the BV/TV in the DMM and CATR groups were higher than those in the Sham group.

The Tb.Th results were shown in Fig. 3(C). At 4 weeks, Tb.Th in the ACL-T and CATT groups were significantly lower than that in the Sham and DMM groups ([ACL-T vs sham], \( P = 0.014, 95\%CI \ [–0.036 to –0.003] \)); [ACL-T vs DMM], \( P = 0.037, 95\%CI \ [0.001–0.034] \)); [CATT vs Sham], \( P = 0.002, 95\%CI \ [–0.041 to –0.008] \)); [CATT vs DMM], \( P = 0.005, 95\%CI \ [0.006–0.039] \)). At 6 weeks, Tb.Th in the DMM group was significantly higher than that in the Sham and ACL-T groups ([DMM vs Sham], \( P = 0.011, 95\%CI \ [0.005–0.045] \)); [DMM vs ACL-T], \( P = 0.011, 95\%CI \ [0.004–0.043] \)). Although there was no significant difference, Tb.Th in the CATT group was higher than that in the Sham group.

There was no significant difference in Tb.N and Tb.Sp between the groups (supplementary results).

**Increased joint instability promotes superficial articular cartilage degeneration**

The results of safranin-O/fast green staining were shown in Fig. 4(A). Surface fibrillation and cartilage destruction in the ACL-T group were confirmed in 5/6 samples at 4 weeks and in 5/6 samples at 6 weeks. In addition, no noticeable superficial cartilage destruction was observed in the other groups. Although there was no significant difference, the OARSI score of the ACL-T group was higher than that of the other groups at 4 weeks [Fig. 4(B)]. Similarly, there was no significant difference in OARSI score at 6 weeks [Fig. 4(B)].

The results of immunohistochemical staining of Coll X were shown in Fig. 5(A). At 4 weeks, the positive cell rate of Coll X in the ACL-T and DMM groups was higher than that in the Sham group [Fig. 5(B)] ([ACL-T vs Sham], \( P < 0.001, 95\%CI \ [8.195–37.338] \)); [DMM vs Sham], \( P = 0.002, 95\%CI \ [6.452–35.595] \)). At 6 weeks, the Coll X-positive cell rate in the ACL-T and DMM groups was higher than that in the Sham group, and that in the CATT group was significantly lower than that in the ACL-T group [Fig. 5(B)] ([ACL-T vs Sham], \( P = 0.002, 95\%CI \ [5.983–33.025] \)); [DMM vs Sham], \( P = 0.022, 95\%CI \ [1.691–28.733] \); [CATT vs ACL-T], \( P = 0.008, 95\%CI \ [–29.272 to –3.488] \)].

**Subchondral bone remodeling differences between models**

The results of TRAP staining were shown in Fig. 6(A). Regarding the activity of osteoclasts in subchondral bone, the Oc.S/BS in the ACL-T group was higher than that in the other groups at 4 weeks [Fig. 6(B)] ([ACL-T vs Sham], \( P < 0.001, 95\%CI \ [4.087–13.863] \)); [ACL-T vs CATT], \( P = 0.015, 95\%CI \ [–10.648 to –0.872] \)); [ACL-T vs DMM], \( P = 0.001, 95\%CI \ [–12.395 to –2.619] \)); [ACL-T vs CATT], \( P = 0.001, 95\%CI \ [–12.433 to –2.657] \)). However, there was no significant difference in Oc.S/BS between the groups at 6 weeks [Fig. 6(B)].

The results of immunohistochemical staining of Osterix were shown in Fig. 7(A). At 4 weeks, the positive cell rate of Osterix in the DMM and CATR groups was higher than that in the Sham group, and that in the CATT group was significantly higher than that in the
ACL-T group [Fig. 7(B)] (DMM vs Sham), $P = 0.004$, 95%CI [4.743–31.480]; (CATR vs Sham), $P = 0.001$, 95%CI [7.605–34.342]; (CATR vs ACL-T), $P = 0.021$, 95%CI [1.748–28.485]). However, at 6 weeks, there was no significant difference in the positive cell rate of Osterix between the groups [Fig. 7(B)].

Discussion

This study examined the difference between articular cartilage degeneration and subchondral bone changes in four models. CATT and CATR models have been established to control joint instability. Chondrocyte hypertrophy was observed in the ACL-T and DMM groups; however, suppressed in the CATT and CATR groups. Changes in subchondral bone were less affected by the increase or decrease in joint instability. The resorption of subchondral bone was promoted in the ACL-T and CATT groups in which ACL deficiency. On the other hand, in the DMM and CATR groups in which meniscal dysfunction occurred, bone formation of the subchondral bone was promoted.

In this study, ACL-T and DMM models with joint instability and CATT and CATR models with suppressed joint instability were used. Soft x-ray analysis showed that the CATT group suppressed the joint instability produced by the ACL-T group (Fig. 2), and that the CATR group suppressed joint instability produced by the DMM group (Fig. 2). In addition, lateral deviation of the medial meniscus was observed in the DMM and CATR groups. These results suggest that the CATR group suppresses joint instability, but increases lateral deviation of the medial meniscus, similar to the DMM group, and the meniscus dysfunction remains.

It has been reported that articular cartilage degeneration depends on the magnitude of joint instability. Adebayo et al. and Glasson et al. had reported that the DMM model has milder joint instability and slower articular cartilage degeneration than the ACL-T model. In addition, our colleague reported that articular cartilage degeneration is delayed by suppressing joint instability that occurs in the ACL-T model. Out histological results confirmed articular cartilage degeneration in the ACL-T group at 4 and 6 weeks. There was no significant difference in the OARSI score, but the CATT, DMM, and CATR groups had mild articular cartilage degeneration. The results of the present study were milder than those of cartilage degeneration reported in previous studies. One reason for this may be the strain of the mice. In our study using the same ICR mice as in the present study, cartilage degeneration in the ACL-T model was mild as in the present study. Therefore, the results of this study may have been influenced by the strain of the mice. The chondrocyte hypertrophy characteristic of early articular cartilage degeneration was significantly increased in the ACL-T and DMM groups, compared to the Sham group at 4 and 6 weeks. However, there was no significant difference between the CATT and CATR groups compared to the Sham group. These results support those of previous studies, which suggest that increased joint instability causes articular cartilage degeneration. Our comparison of different models suggested that joint instability was involved in superficial articular cartilage degeneration.

Subchondral bone changes are important for the onset of OA. Abnormal subchondral bone remodeling is essential for OA pathology. Previous reports that had induced subchondral bone remodeling in a single model reported that the ACL-T model causes bone loss and that the DMM model increases BV/TV early after
the intervention. Interestingly, suppression of joint instability inhibited chondrocyte hypertrophy, but had no effect on subchondral bone changes. Specifically, different changes were observed in the ACL-T and CATT groups, in which transected ACL, and in the DMM and CATR groups, in which transected MMTL. The BV/TV decreased in the ACL-T and CATT groups with ACL disconnection. Nomura et al. had reported that reducing the mechanical stress on joints reduced the BV/TV. Therefore, we speculated that in the ACL-T and CATT groups, joint instability in the anterior-posterior direction caused the decreased BV/TV. In addition, osteoclast activity by TRAP staining was increased in the Oc.S/BS in the ACL-T group. Therefore, we inferred that ACL amputation causes joint instability and distributes mechanical stress to the knee joint by dispersing the contact area, leading to osteoclast activation and BV/TV reduction. In contrast, BV/TV increased in the DMM and CATR groups in which lateral deviation of the medial meniscus was confirmed by MMTL transection. BV/TV and Tb.Th increase when an axial compression load is applied to the tibia. Thus, we

Fig. 5

Comparison of Col X positive cell rates. (A) Representative Col X-stained image of each group. (B) At 4 weeks, the positive cell rate of the ACL-T and DMM groups was higher than that of the Sham group. Similarly, at 6 weeks, the positive cell rate of the ACL-T and DMM groups was higher than that of the Sham group. The positive cell rate in the CATR group was lower than that in the ACL-T group. Data are presented as the mean with 95% CI. Scale bar: 100 μm.
speculated that the increased BV/TV and Tb.Th in the DMM and CATR groups may have been caused by the increased compressive load on the medial tibia due to lateral deviation of the meniscus. In addition, the Osterix-positive cell ratio was higher in the DMM and CATR groups. Therefore, we inferred that the lateral deviation of the medial meniscus promoted osteoblast differentiation and contributed to bone formation.

Summarize, it was suggested that the suppression of joint instability inhibited articular cartilage degeneration by suppressing chondrocyte hypertrophy. However, the changes in the subchondral bone showed different characteristics in the ACL transected group and the MMTL transected group. Therefore, we inferred that the lateral deviation of the medial meniscus promoted osteoblast differentiation and contributed to bone formation.

The results of TRAP staining. (A) Representative TRAP-stained image of each group. (B) The results of Oc.S/BS. At 4 weeks, Oc.S/BS in the ACL-T group was higher than that in the other groups. No significant difference was observed at 6 weeks. Data are presented as the mean with 95% CI. Scale bar: 100 μm.

The main limitations of this study are the following two points. First, the joint instability was only evaluated under static conditions. Therefore, it is necessary to evaluate dynamic joint instability by analyzing the difference in joint instability during walking of mice by motion analysis. Second, the observed articular cartilage degeneration is mild, and the difference in articular cartilage degeneration and subchondral bone changes between the models may be due to the difference in progression rate. Thus, future studies should investigate the difference in articular cartilage degeneration and subchondral bone changes of each model by performing longer-term verification.
In conclusion, the results of this study suggested that joint instability promotes chondrocyte hypertrophy and contributes to articular cartilage degeneration. The results also suggested that ACL or meniscus dysfunction may have different effects on subchondral bone changes.

Contributions
All authors approved the final submitted manuscript.
Study design: KA, KM, NK, and TK.
Data collection, Histological analysis: KA, KT, YO, KO, SN and SE.
Manuscript composition KA, TK.

Conflict of interest
All authors have no conflicts of interest related to the manuscript.

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