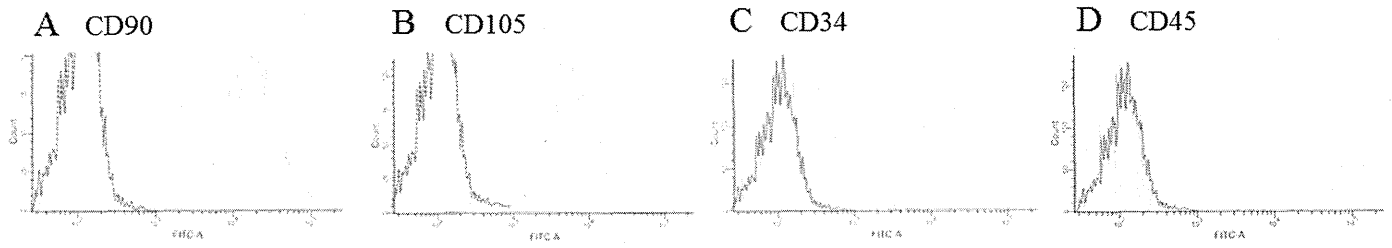
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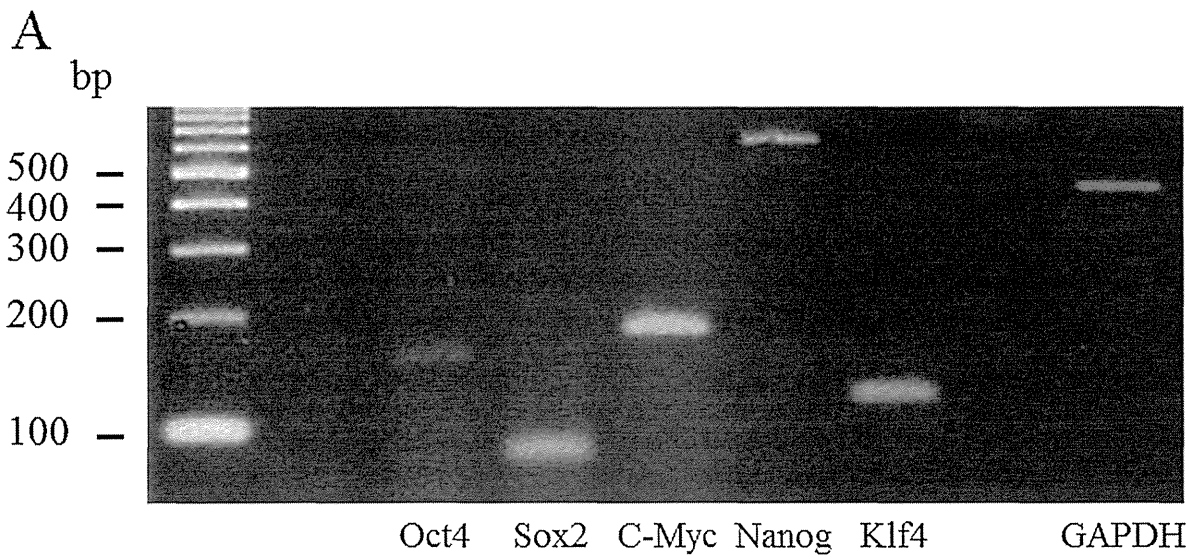
B CD105
C CD34
D CD45


A
bp
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300
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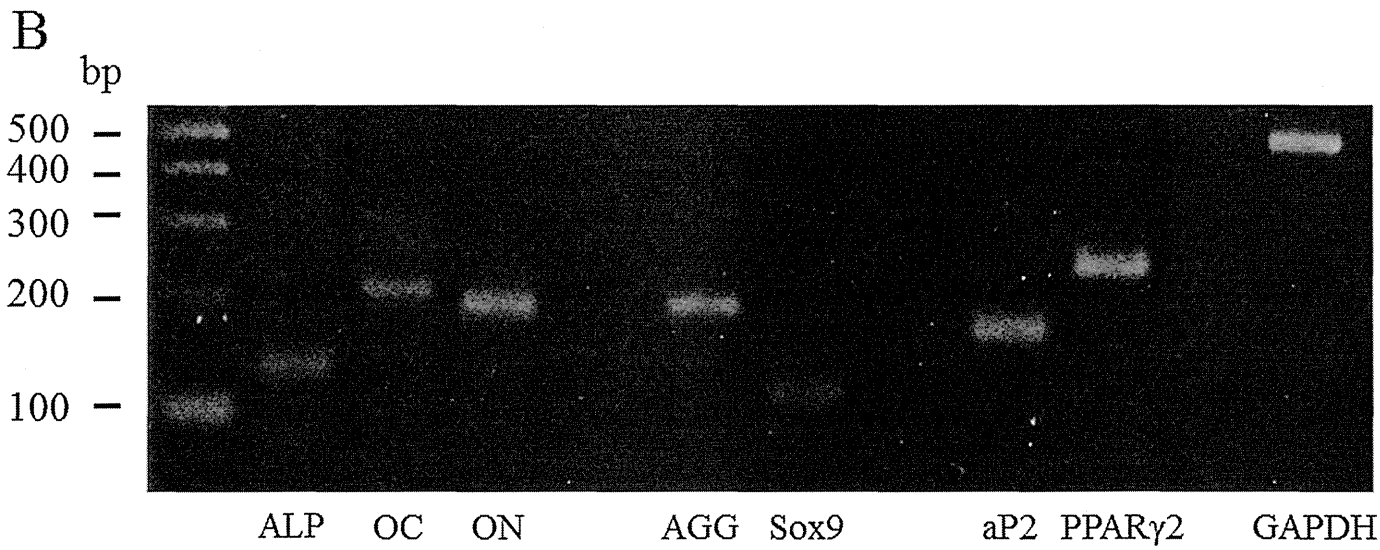
100
Oct4
Sox2
C-Myc
Nanog
Klf4
GAPDH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OC
ON
AGG
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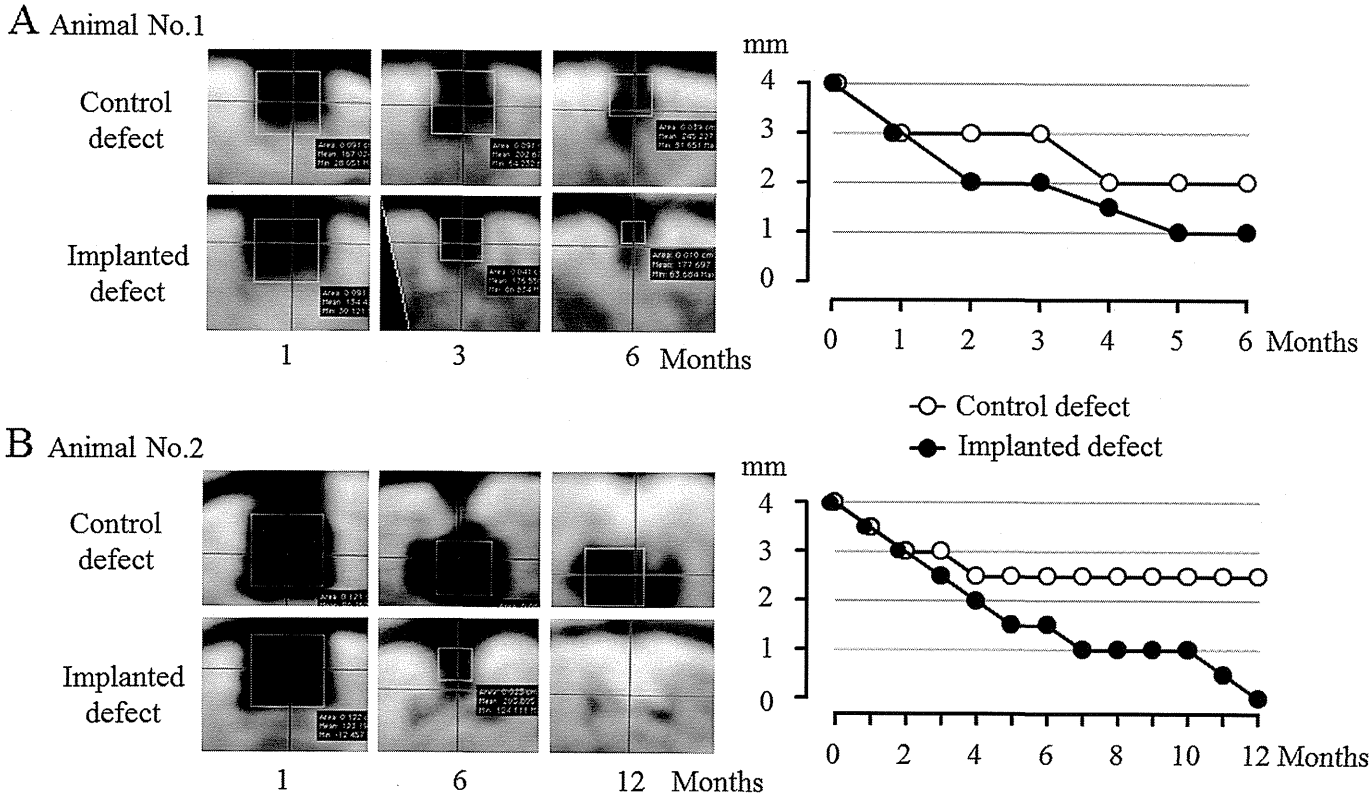
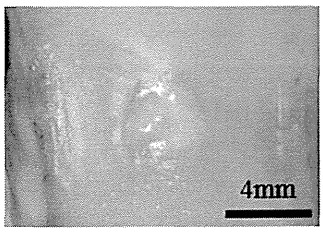
aP2
PPARγ2
GAPDH

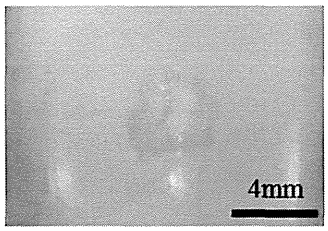

A Animal No.1
Control defect
Implanted defect
1
3
6 Months
mm
4
3
2
1
0
0 1 2 3 4 5 6 Months
Control defect
Implanted defect
B Animal No.2
Control defect
Implanted defect
1
6
12 Months
mm
4
3
2
1
0
0 2 4 6 8 10 12 Months

6 months after the surgery (Animal No.1)

A Implanted defect

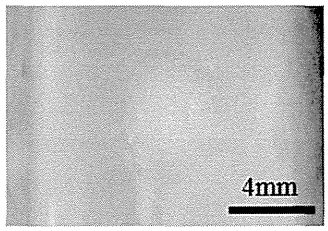


B Control defect

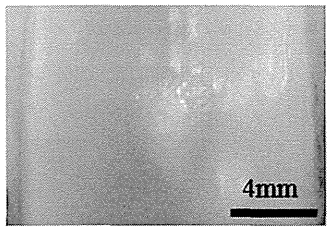


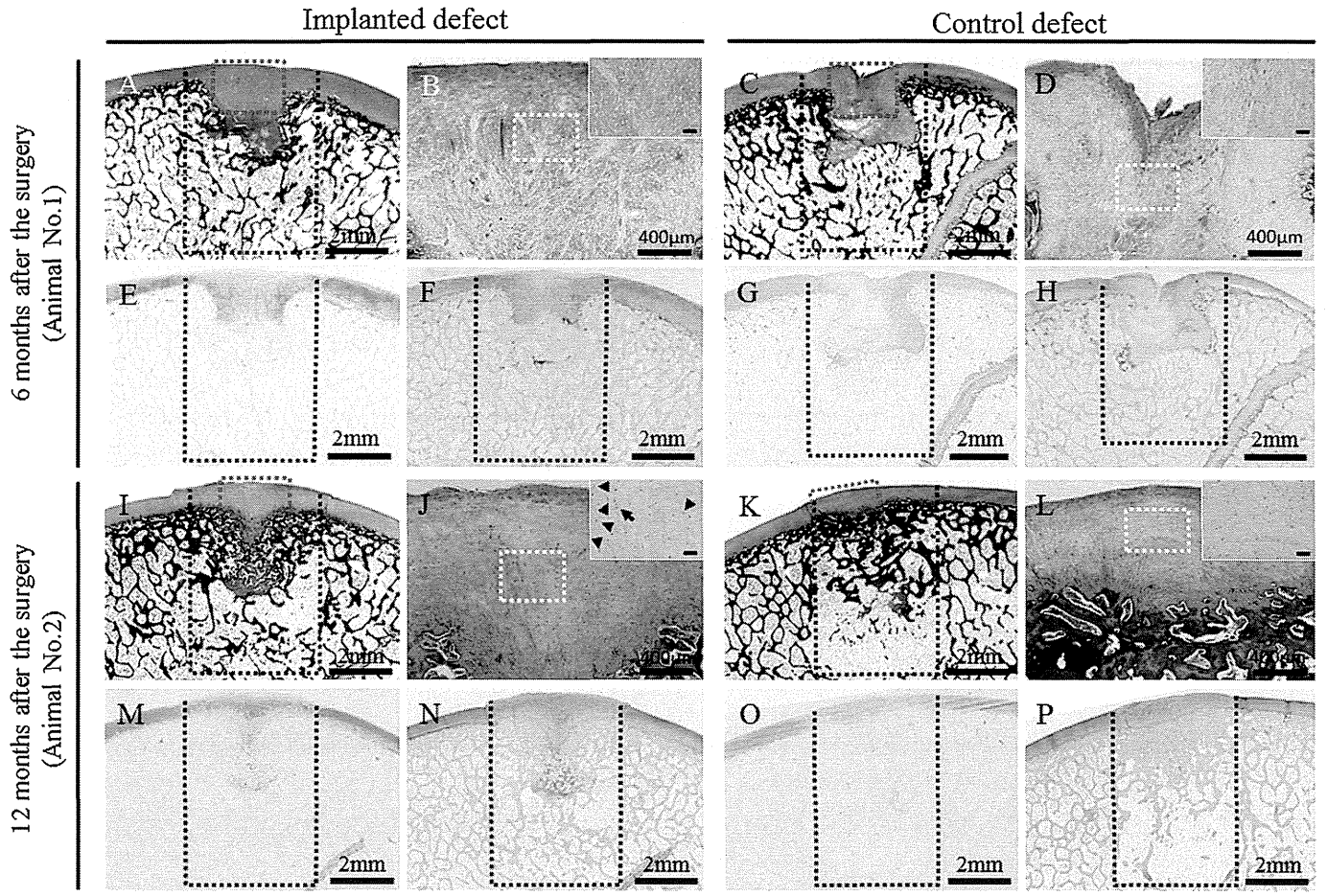
12 months after the surgery (Animal No.2)

C Implanted defect



D Control defect




Implanted defect
Control defect
6 months after the surgery (Animal No.1)
12 months after the surgery (Animal No.2)
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
2mm
400μm

Additional files provided with this submission:

Additional file 1. Surgical procedure, CT images, macroscopic findings of the articular surface, and histopathology of osteochondral defects in animal no. 3. Figure S1, surgical procedure: A columnar construct (6 mm in diameter and 8 mm in height) composed of about 1,150 spheroids of AT-MSCs (A). An elliptic cylindrical osteochondral defect in each groove (B). Two constructs were autografted into the defect of the right hind limb (C). No implantation was in the left limb (B). Figure S2, CT images after the surgery: One cross section of the multi-planar reconstruction images 1, 6, and 12 months after the surgery in animal no. 3. In the implanted site, the radiopaque area gradually progressed and filled throughout the osteochondral defect after 12 months. However, in the control site, the spread of the radiopaque area was limited in the shallow layer, and no bone formation was in the deep layer. Figure S3, macroscopic findings of the articular surface: The surface was completely covered with abundant cartilaginous white tissues. The boundary to the surrounding normal cartilage was not different between the implanted site (A) and the control site (B). Figure S4, histopathology of osteochondral defects: At the implanted site, the restored subchondral bone was covered by mixture of hyaline/fibrocartilage, in which the clusters (arrowhead) and columnar clusters (arrow) of the cells were seen (A, B, C, D). In the control site, the surface was irregular, and the large fibrous tissue was presented in the subchondral (area with no bone at the bottom half of the defect (E, F, G, H)). Black dotted lines indicate the areas of osteochondral defects immediately after the surgery. Images B and F are high-power fields of the red dotted square in images A and E, respectively. The small images in sections B and F are high-power fields of white dotted squares in the respective images. The bars in the small images indicate 50 μm (469kb)
http://www.josr-online.com/content/supplementary/s13018-015-0173-0-s1.pdf

Additional file 2. ICRS gross grading scale and histological grading scale in animal no. 3. Table S1, ICRS gross grading scale. Table S2, ICRS histological grading scale (11kb)
http://www.josr-online.com/content/supplementary/s13018-015-0173-0-s2.xlsx

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RESEARCH ARTICLE Open Access

Detection of early cartilage deterioration associated with meniscal tear using T1ρ mapping magnetic resonance imaging

Hirokazu Matsubara[1], Ken Okazaki[1*], Yukihisa Takayama[2], Kanji Osaki[1], Yoshio Matsuo[2], Hiroshi Honda[2] and Yukihide Iwamoto[1]

Abstract

Background: In patients with degenerative meniscal tears, subclinical cartilage degeneration may be present even if gross morphological changes are not evident. The aim of this study was to detect occult cartilage degeneration using T1ρ MRI mapping in patients with meniscal tears without obvious radiographic osteoarthritis (OA).

Methods: A total of 22 subjects with degenerative meniscal tears in the early stages of osteoarthritis [Kellgren-Lawrence (KL) grade of 0–2] and 19 healthy subjects as the control group were examined. The femoral condyle was divided into four 30° wedges (−30°–0° anteriorly, 0°–30°, 30°–60° and 60°–90° posteriorly), and each area of cartilage was further divided into superficial and deep layers of equal thickness. The tibial side was divided into anterior and posterior areas with superficial and deep layers in each. The mean T1ρ values (ms) in each area were calculated.

Results: On the femoral side, T1ρ values of the superficial and deep regions (−30°–0°, 0°–30° and 30°–60°) in the meniscal tear group were significantly higher than those in the control group [superficial (−30°–0°): 49.0 ± 4.0 (meniscal tear group) vs 45.1 ± 2.1 (control group), deep (−30°–0°): 45.2 ± 3.3 vs 39.5 ± 5.0, superficial (0°–30°): 54.5 ± 5.3 vs 47.4 ± 5.7, deep (0°–30°): 46.8 ± 4.0 vs 40.7 ± 6.3, superficial (30°–60°): 50.5 ± 3.1 vs 47.1 ± 5.7]. On the tibial side, the meniscal tear group had significantly higher T1ρ values superficially in both anterior and posterior regions compared with the control group [superficial (anterior): 52.0 ± 4.3 vs 46.7 ± 5.4, superficial (posterior): 53.1 ± 5.1 vs 46.0 ± 4.9]. Moreover, these significant differences were observed when comparing patients in the meniscal tear group with KL grades of 0 or 1 and the control group.

Conclusions: Our study suggested that early biochemical changes in cartilage associated with degenerative meniscal tears occur first in the superficial zones in areas of contact during slight flexion. Characterising the early relationship between cartilage degeneration and degenerative meniscal tears using T1ρ MRI mapping may be of clinical benefit and provide further evidence linking meniscal injury to OA.

Keywords: Cartilage degeneration, Meniscus, Osteoarthritis, Magnet resonance imaging (MRI), T1ρ MRI

Background

Despite the high prevalence of meniscal tears, the role of meniscal injury in the pathogenesis of articular cartilage degeneration is poorly understood [1]. The menisci play an important role in knee joint stability, joint lubrication and shock absorption and help to maintain the integrity of the articular cartilage [2,3]. A number of studies have shown an association between knee osteoarthritis (OA) and meniscal damage [4-8]. These findings support the theory that disruption and excision of the meniscus contribute to cartilage degeneration or progression of OA. We have encountered several cases in which hydrarthrosis with subsequent progressive cartilage degeneration occurred after meniscectomy, although obvious morphological changes had not been detected during preoperative evaluation using radiography and conventional magnetic resonance imaging (MRI) or during intraoperative arthroscopy. We hypothesise that subclinical

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cartilage degeneration occurs after the occurrence of a degenerative meniscal tear, even if gross morphological changes are not evident in the cartilage. Meniscectomy is commonly performed on a patient with knee joint symptoms despite being associated with a mild OA such as that of Kellgren and Lawrence grade 2. Therefore, it would be a clinical benefit if the surgeon could pre-operatively evaluate occult cartilage degeneration in patients with a meniscal tear. In addition, characterising the early relationship between cartilage degeneration and meniscal damage may provide further evidence linking meniscal injury to OA.

Early stages of OA are primarily associated with loss of proteoglycans (PG), changes in the water content and minor structural changes in collagen [9]. MRI techniques have the potential to detect such biochemical changes in the composition of joint articular cartilage non-invasively [10,11]. Specifically, T1ρ mapping has been shown to be sensitive to changes in PG loss in cartilage and has attracted attention as a non-invasive and a quantitative means of detecting biochemical changes in cartilage degeneration prior to morphological or clinical changes [10-12]. Previous studies have demonstrated that T1ρ values are elevated in the cartilages of OA patients when compared with corresponding healthy subjects [13,14]. T1ρ values are correlated with the severity of macroscopic cartilage damage and histologically-assessed PG content in the cartilages of patients with OA or rheumatoid arthritis [15]. Therefore, we used T1ρ mapping to detect early cartilage degeneration associated with degenerative meniscal tears.

The purpose of our study was (1) to detect occult cartilage degeneration in patients with degenerative meniscal tears using T1ρ MRI mapping, (2) to investigate the distribution of T1ρ values in the superficial and deep layers of adjacent femoral and tibial cartilages and (3) to characterise the relationship between early cartilage degeneration and degenerative meniscal tears.

Methods

Subjects

This study was granted Institutional Review Board approval at Kyushu University and complied with the Ethical Committee Standards (approval number: 23–75). All subjects provided written informed consent prior to the study. The subjects were recruited from the patients who visited our hospital due to knee joint symptoms. Table 1 shows the baseline characteristics of the subjects included in the study. Anthropometric measurements, including height and weight, were collected, and body mass index (BMI) was calculated for all participants. MRI and radiographic examination was performed on all subjects. Meniscal lesions were graded based on MRI images using the following grading system: grade 0, normal meniscus; grade 1, increased signal intensity of the meniscus without evidence of a tear; grade 2, small radial meniscal tear; grade 3, non-displaced single meniscal tear; grade 4, non-displaced complex meniscal tear; grade 5, meniscal tear with displaced component; and grade 6, macerated meniscal tear [16]. Patients with meniscal changes scoring 2–6 on MRI were assigned to the meniscal tear group. Femoro-tibial angle (FTA) values were calculated based on radiographs, and radiographic severity of OA was determined according to the Kellgren and Lawrence (KL) grading system. The meniscal tear group comprised 22 subjects: Fifteen males and seven females ranging in age from 32 to 73 years (mean, 56.4 ± 12.8 years) with KL grades between 0 and 2. Only patients with frequent symptoms, defined as pain in or around the knee on most days for at least 1 month over the past 12 months and a positive McMurray test, were investigated. Subjects who had severe OA or KL grades more than 3 were excluded, as were subjects with a ligament injury and/or cartilage injury on MRI. To categorise the severity of OA, subjects in the meniscal tear group were further divided into two subgroups: 'KL normal' with a KL grade of 0 or 1 (n = 12) and 'KL mild OA' with a KL grade of 2 (n = 10). The meniscal tear group consisted of 20 medial and two lateral meniscal tears. The control group comprised 19 healthy individuals, all males and ranging in age from 28 to 54 years (mean, 39.0 ± 7.2 years) without any clinical symptoms of OA or other knee injuries. All controls were only enrolled in the study if they had a KL grade of 0, indicating no signs of radiographic OA.

Table 1 Baseline characteristics and radiographic parameters of meniscal tear group and control group

	Meniscal tear group	Control group	*P* value
Characteristic			
Participants, no	22	19	
Age, mean ± SD, years	57.0 ± 14.1	39.0 ± 7.2	< 0.05
BMI, mean ± SD, kg/m^2	24.4 ± 2.6	23.9 ± 2.1	0.31
Radiographic parameter			
FTA, mean ± SD, °	177.0 ± 2.6	176.7 ± 0.8	0.64
KL grade, no			
Grade 0	4	16	
Grade1	8	3	
Grade2	10	0	

BMI body mass index, FTA femoro-tibial angle, KL Kellgren and Lawrence.

Magnetic resonance imaging protocols

MRI was performed on a 3-Tesla system (Achieva 3.0 T, Quasar Dual, Philips Healthcare, Best, the Netherlands) using an 8-channel phased-array knee coil. Sagittal fat-suppression turbo spin echo T2-weighted images

(FS-T2WI) were obtained using the following parameters: Repetition time/echo time (TR/TE) = 4,675/71 ms, field of view (FOV) = 140 × 140 mm, matrix = 400 × 400, slice thickness = 3 mm, slice gap = 0 mm, flip angle = 90°, bandwidth = 31.54 Hz/pixel, number of slices = 26 and total scan time = 3 min 33 s. FS-T2WI was used as an anatomical reference.

Two-dimensional (2D)-Sagittal T1ρ mapping was calculated from T1ρ-prepared images using the fast field echo technique. The imaging parameters were as follows: TR/T = 4.7/2.4 ms, FOV = 140 × 140 mm, matrix = 320 × 320, slice thickness = 3 mm, slice gap = 0 mm, flip angle = 35°, bandwidth = 31.54 Hz/pixel, spin-lock pulses = 20/1/40/60/80 ms, spin-lock pulse frequency = 500 Hz, number of slices = 26 and total scan time = 16 min 15 s. We used a low flip angle, but it did not affect T1ρ contrast, since we used 6,000 ms of shot intervals between each slice acquisition and filled the k-space using low-high ordering. T1ρ mapping was produced with Philips Research Integrated Development Environment (PRIDE) software written in Interactive Data Language (IDL 6.3, ITT Inc. Boulder, CO, USA) and was used in the quantitative assessment.

Imaging assessment of T1ρ maps

Mean T1ρ values and standard deviations were calculated by two orthopaedic surgeons (H.M. and K.O., with nine and eight years' experience, respectively) using the 'Medical Image Processing, Analysis, and Visualization' software (MIPAV, Biomedical Imaging Research Services Section, Center for Information Technology, National Institutes of Health, Bethesda, MD, USA) (Figure 1a). On the femoral side, regions of interest (ROIs) in the articular cartilage were divided into anterior and posterior parts. The posterior part was further divided into three 30° wedges since the medial femoro-tibial contact point shifts posteriorly until 60° of flexion [17,18], which increases the risk of meniscal injury more than in extended positions. Furthermore, we distinguished superficial and deep layers of equal thickness in all areas (Figure 1b). ROIs of the articular cartilage on the tibial side were divided into anterior and posterior areas, and

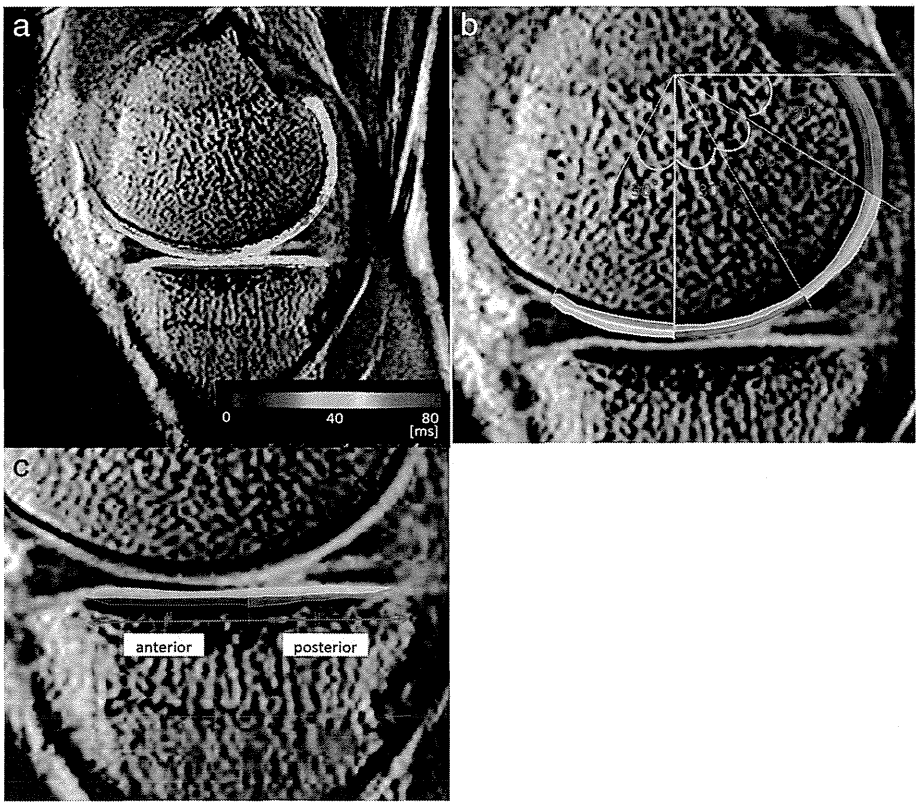

Figure 1 Assessment of T1ρ values in the articular cartilage. a. Colored T1ρ mappings of femoral and tibial articular cartilage. **b**. Regions of interest (ROIs) were divided into eight regions on the femoral articular cartilage; superficial (–30°–0°): yellow; deep (–30°–0°): cyan; superficial (0°–30°): red; deep (0°–30°): orange; superficial (30°–60°): yellow-green; deep (30°–60°): green; superficial (60°–90°): blue; deep (60°–90°): purple. **c**. ROIs were divided into four regions on the tibial cartilage; superficial (anterior): orange; deep (anterior): red; superficial (posterior): yellow-green; deep (posterior): blue.

superficial and deep layers were analysed separately (Figure 1c). In addition, anterior and posterior horn of the meniscus on the same MRI slice was also analyzed. Three adjacent slices at the centre of the medial or lateral compartment in the sagittal plane, respectively, were analysed for each subject. After analysing the ROIs for each of the 14 areas (femoral side, eight areas; tibial side, four areas; meniscus, two areas) on each of the three slices, mean T1ρ values and standard deviations were used for the statistical analyses.

Statistical analyses

All data are expressed as the mean ± SD. A Mann–Whitney U test was performed to compare T1ρ values for each area on the femoral and tibial articular cartilage between the meniscal tear group and the control group and between genders. Analysis of variance (ANOVA) and a post hoc comparison using Tukey's honest significant difference test were used to assess differences in T1ρ values for each area on the femoral and tibial articular cartilage between the control, the KL normal and KL mild OA groups. In addition, Pearson's correlation analysis was applied to study the relationship between patient age and T1ρ values for each area on the femoral and tibial articular cartilage of both the meniscal tear and the control groups. The intra-class correlation coefficient (ICC) was used to describe inter-observer agreement for the measurement of the T1ρ values. ICCs close to 1.0 indicated good agreement between the two observers. All statistical tests were performed with the JMP software version 9.0 (SAS Institute, Cary, NC, USA). A P-value <0.05 was considered statistically significant for each two-tailed analysis.

Results

T1ρ values in each area of the articular cartilage on the femoral condyle and tibial plateau are summarized in Table 2 and Table 3, respectively. For the femoral condyle, T1ρ values in the meniscal tear group were significantly higher than those in the control group in both the superficial and deep regions of the −30°–0° wedge and the 0°–30° wedge, and in the superficial region of the 30°–60° wedge (Table 2 and Figure 2a). With regard to the differences in OA severity between the control group and the two subgroups, a statistically significant difference was observed in both the superficial and deep regions of the −30°–0° wedge and the 0°–30° wedge between the control group and the KL normal group and between the control group and the KL mild OA group. However, no significant differences were observed between the two subgroups in the meniscal tear group (Table 2 and Figure 2b). No statistically significant differences were observed between the genders as well (Table 2 and Figure 2c).

For the tibial plateau, the meniscal tear group had significantly higher T1ρ values than the control group in both the anterior and posterior regions in the superficial areas (Table 3 and Figure 3a). With regard to the differences between the control group and the two subgroups in the meniscal tear group, a statistically significant difference was observed in the superficial areas of both the anterior and posterior regions between the control group and the KL normal group, and between the control group and the KL mild OA group. However, no statistically significant differences were observed between the two subgroups in the meniscal tear group or between the genders, similarly to the findings related to the femoral condyle (Table 3 and Figure 3b and c). The T1ρ values (ms) of the posterior horn of meniscus were significantly higher in the meniscal tear group than those in the control group (44.2 ± 3.5 vs 35.6 ± 2.1, respectively, $p < 0.01$). There were no significant differences in T1ρ values of the anterior horn of meniscus between in the meniscal tear group and the control group (36.0 ± 2.1 vs 34.4 ± 3.1, respectively).

No significant correlations were observed between age and T1ρ values at any regions of both the femoral condyle and the tibial plateau in the meniscal tear group (Figure 4a and b), but slight or moderate correlations were observed between these in the control group (Figure 4c and d). The intra-class correlation coefficient (ICCs) of the T1ρ values between the two observers was 0.93 (95% CI = 0.84–0.95), indicating good agreement between the observers.

Discussion

In the present study, we evaluated sub-regional and layer-specific T1ρ values of femoro-tibial articular cartilage in patients with meniscal tears with no or mild radiographic OA and compared them with those of healthy subjects. Higher T1ρ values were observed at the distal area of the femoral condyle and the tibial plateau in the meniscal tear group, even in the subgroup with no radiographic OA. Hence, the study suggested the association between meniscal tears and occult cartilage damage.

However, this study had several limitations. First, the mean age was different between the meniscal tear group and the control group, and the average age of the subjects in the meniscal tear group was relatively high. It was challenging to recruit age-matched control subjects from volunteers. In fact, there were weak or moderate correlations between age and T1ρ values in articular cartilage in the control group. However, no correlations were observed between age and T1ρ values in the meniscal tear group. Patients less than 50 years old with

Table 2 T1ρ values (ms) for articular cartilage on the femoral condyle

	Superficial (−30°-0°)	Deep (−30°-0°)	Superficial (0°-30°)	Deep (0°-30°)	Superficial (30°-60°)	Deep (30°-60°)	Superficial (60°-90°)	Deep (60°-90°)
Meniscal tear group	49.0 ± 4.0	45.2 ± 3.3	54.6 ± 5.3	46.8 ± 4.0	50.5 ± 3.1	52.2 ± 3.6	45.4 ± 3.1	46.2 ± 3.4
Control group	45.1 ± 2.1	39.6 ± 5.0	47.4 ± 5.7	40.7 ± 6.3	47.1 ± 5.7	47.8 ± 7.6	42.5 ± 5.8	42.9 ± 6.4
P-value	<0.05	<0.05	<0.05	<0.05	<0.05	0.08	0.29	0.15
Comparison between the OA severity groups								
Normal (n = 12)	47.7 ± 3.8	47.1 ± 4.0	53.2 ± 6.3	46.1 ± 4.2	49.8 ± 3.5	51.9 ± 3.5	45.0 ± 4.0	46.3 ± 4.2
Mild OA (n = 10)	48.2 ± 3.3	48.9 ± 4.2	56.0 ± 4.4	47.1 ± 4.0	51.1 ± 3.5	52.5 ± 4.0	45.0 ± 2.3	45.8 ± 2.5
P-value	0.88	0.72	0.18	0.87	0.62	0.84	0.87	0.71
Comparison between genders								
Male (n = 15)	47.7 ± 3.7	45.3 ± 3.5	54.6 ± 5.2	46.9 ± 4.1	50.1 ± 3.5	52.6 ± 4.2	46.0 ± 3.6	46.3 ± 4.1
Female (n = 7)	48.2 ± 4.5	46.7 ± 4.1	54.7 ± 6.0	46.8 ± 4.0	51.4 ± 2.5	51.5 ± 2.4	44.0 ± 1.9	46.2 ± 2.0
P-value	0.25	0.32	0.33	0.80	0.29	0.53	0.32	1.00

Table 3 T1ρ values (ms) for articular cartilage on the tibial plateau

	Superficial (anterior)	Deep (anterior)	Superficial (posterior)	Deep (posterior)
Meniscal tear group	52.0 ± 4.3	41.3 ± 3.7	53.1 ± 5.1	42.8 ± 4.3
Control group	46.7 ± 5.4	39.6 ± 5.0	46.0 ± 4.9	41.3 ± 4.8
P-value	<0.05	0.28	<0.05	0.55
Comparison between the OA severity groups				
Normal (n = 12)	51.7 ± 4.5	41.6 ± 3.9	52.9 ± 5.5	43.0 ± 4.9
Mild OA (n = 10)	51.8 ± 4.3	40.4 ± 3.3	52.3 ± 4.8	41.6 ± 3.6
P-value	1.00	0.49	0.37	0.22
Comparison between genders				
Male (n = 15)	52.5 ± 5.0	41.6 ± 4.3	55.0 ± 4.7	42.5 ± 4.7
Female (n = 7)	50.6 ± 2.4	40.6 ± 2.3	51.0 ± 5.3	42.4 ± 4.1
P-value	0.70	0.64	0.19	0.97

meniscal tears also showed relatively high T1ρ values. Therefore, the increased T1ρ values in the meniscal tear group were not attributed to the relatively higher age of this group. Second, the magic angle effect may affect the calculation of T1ρ values in articular cartilage, especially in the 30°–60° area. This area is susceptible to the magic angle effect both superficially and deeply since this effect increases T1ρ values relating to the orientation of collagen fibrils in articular cartilage and a static magnetic field [19,20]. Therefore, attention should be paid to results showing that T1ρ values are significantly higher in this area, when comparing different areas in the same

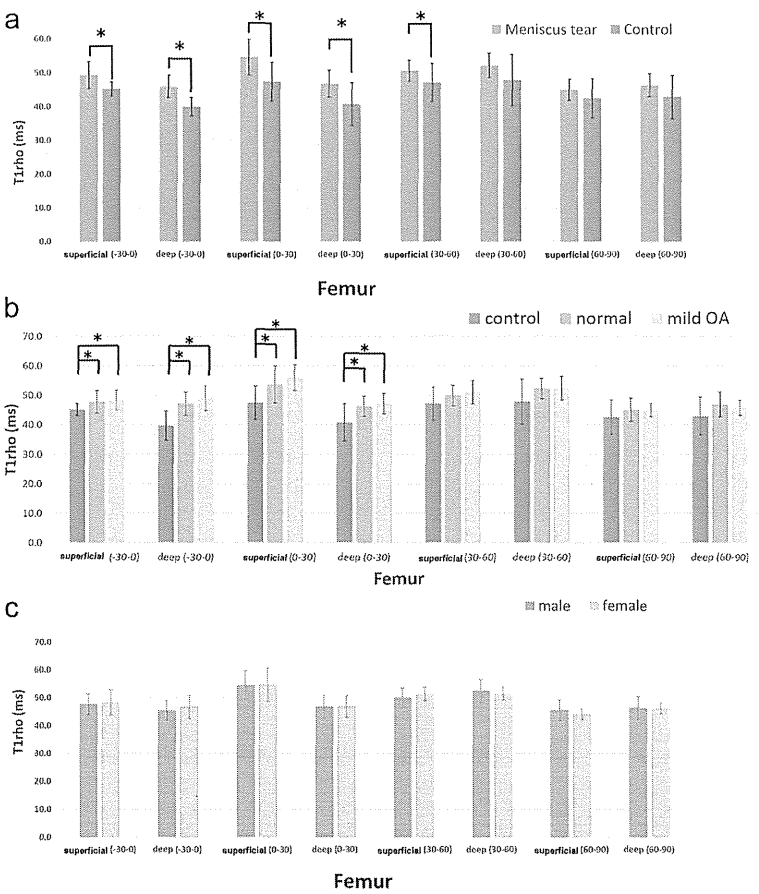

Figure 2 T1ρ values in different cartilage regions of the femoral condyle. a. Meniscal tear and control groups **b**. Normal and mild OA **c**. Males and females. Error bars: 95% confidence interval *: P < 0.05.

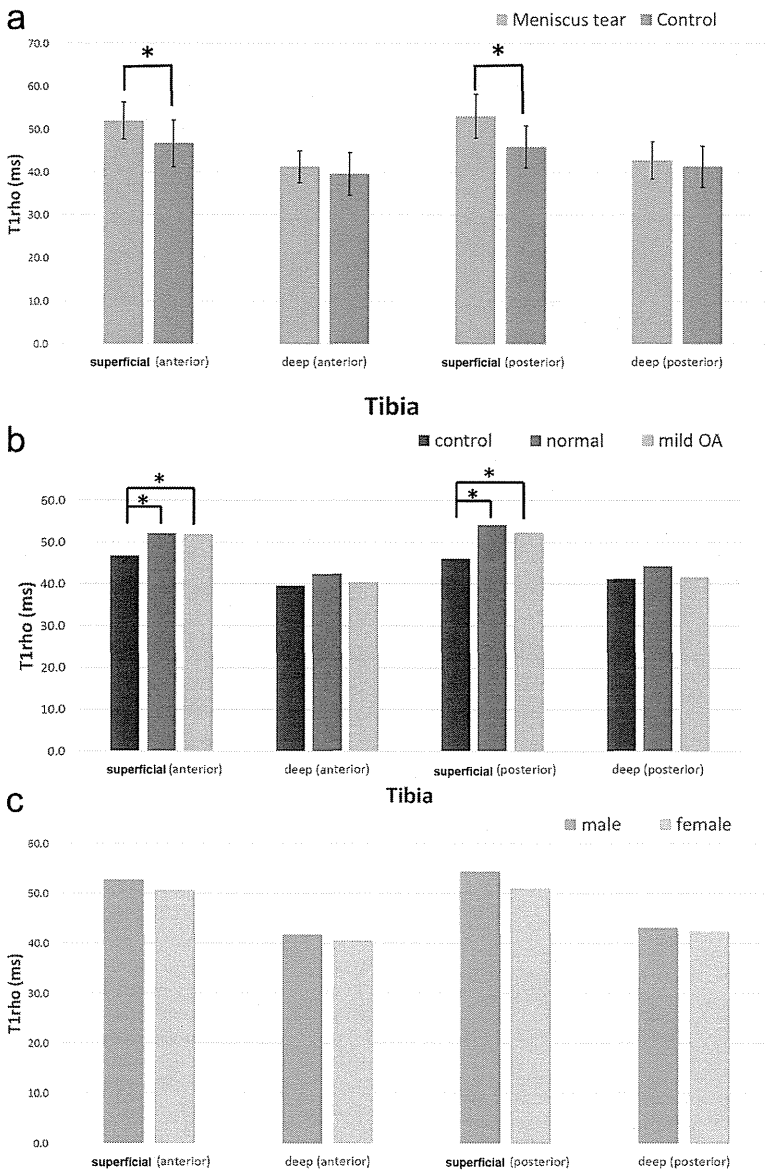

Figure 3 T1ρ values in different cartilage regions of the tibial plateau. a. Meniscal tear and control groups. **b**. Normal and mild OA. **c**. Males and females. Error bars: 95% confidence interval *: P < 0.05.

knee. However, this study compared the same area between groups of subjects, indicating that T1ρ values in both superficial and deep regions of the 30°–60° area in the meniscal tear group were significantly higher than those of the control group. Therefore, there is no doubt that cartilage denaturation had occurred in association with a degenerative meniscal tear in these areas. Third, ROIs were manually drawn around the articular cartilage boundaries to calculate T1ρ values. Factors that might cause miscalculation include the misidentification of articular cartilage boundaries, such as that between the superficial and deep layers. However, ICCs indicated good agreement between the observers, and so any accidental error associated with drawing the ROIs would not significantly affect the conclusions of this study. Finally, the relatively small sample size and the cross-sectional design are other limitations of the study. It was also difficult to evaluate other potential confounders influencing the T1ρ values with multivariable analysis.

Previous studies have evaluated the relationship between cartilage change and OA using a variety of methods. Bassiouni et al. examined the cartilage in OA using phonoarthrography, musculoskeletal ultrasonography and biochemical biomarkers and concluded that phonoarthrography and musculoskeletal ultrasonography could be used as parameters for following up

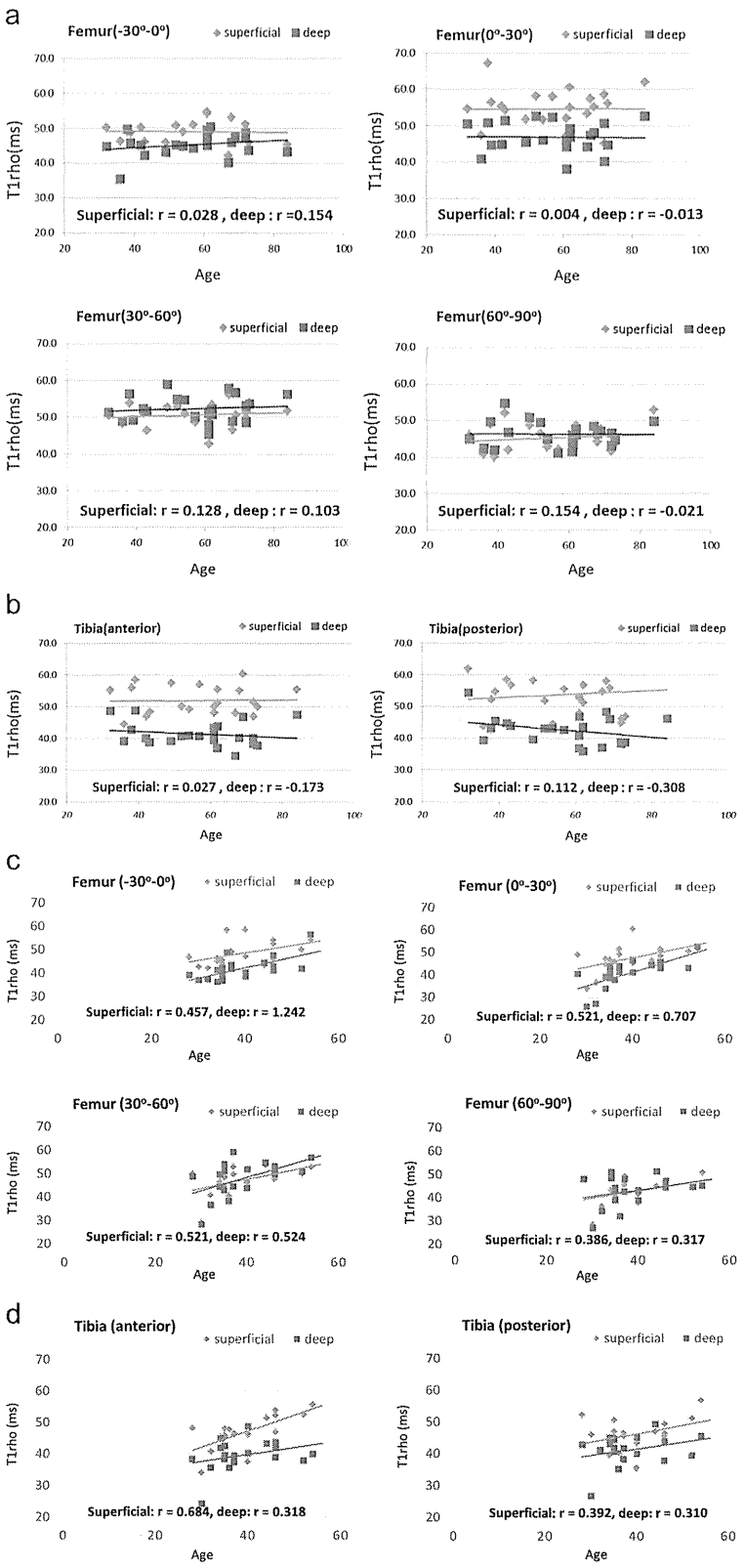

Figure 4 The relationship between age and T1ρ values of different cartilage regions. a. Femoral condyle of the meniscal tear group. **b**. Tibial plateau of the meniscal tear group. **c**. Femoral condyle of the control group. **d**. Tibial plateau of the control group.

cartilage disorders in OA knees [21]. T1ρ-weighted MRI methods have been proposed recently as an attractive potential biomarker to evaluate biochemical changes in the cartilage matrix non-invasively [10,11]. It has been reported that T1ρ relaxation time is sensitive to early biochemical changes in cartilage, especially the PG content [12,15]. In the early stages of OA, PG depletion occurs prior to the minor structural changes of collagen (denatured collagen) [15]. T1ρ MRI mapping is a useful imaging method to detect this PG depletion, and minor changes of denatured collagen may also contribute to the T1ρ signal. Takayama et al. reported that T1ρ mapping is superior to T2 mapping for the assessment of denatured articular cartilage in the early stages of OA, with a capability to assess the severity of cartilage degeneration with good accuracy [22]. This study utilized T1ρ MRI mapping to detect early cartilage damage in patients with degenerative meniscal tears and healthy subjects.

Some investigators have compared T1ρ values of subregions and the whole area of the femoro-tibial cartilage and menisci in patients with OA [23], whereas others investigated the relationship between T2 relaxation values within the superficial zone of the articular cartilage following different types of meniscal degeneration/tear [24]. However, most of these previous studies assessed a limited or region-specific area of the entire joint, such as in one slice of the mid-sagittal plane of the medial and lateral femoral condyles. Although it is known that the severity of degeneration is heterogeneous and focal within the OA joint, there is still a lack of information about the regions of the weight-bearing area in the femoral condyles and the tibia plateaus that are likely to be affected by the early degenerative changes associated with a meniscal tear. Furthermore, superficial and deep layer-specific assessment of the articular cartilage might be more sensitive in terms of detecting early changes in the cartilage macromolecular structures. In the present study, femoral articular cartilage in the meniscal tear group had significantly greater T1ρ values at the distal area, both superficially (−30°–0°, 0°–30° and 30°–60° areas) and in the deep regions (−30°–0° and 0°–30° areas). In tibial articular cartilage, significantly higher T1ρ values were found superficially in both anterior and posterior areas in the meniscal tear group than in the control group. In both femoral and tibial articular cartilage, no significant differences in T1ρ values between the two subgroups of OA severity in the meniscal tear group were observed. This finding suggests that degenerative meniscal tears are associated with occult cartilage damage in the very early stages of OA rather than radiographic OA severity, and supports the previous studies that have reported the direct interaction between meniscal and cartilaginous abnormality [1,8,23,24]. Hence, occult cartilage damage exists in patients with a symptomatic meniscus tear even though there is no evidence of radiographic OA. The meniscus plays an important role in maintaining the integrity of the articular cartilage by reducing the contact impact forces between the articular surfaces. Damage to the meniscus increases the contact peak stress on the femoral and tibial articular cartilage surfaces and alters the biomechanical loading of the joint, putting the patient at increased risk of cartilage degeneration [18,25].

Previous studies using T1ρ or T2 MRI mapping have demonstrated a relationship between meniscal and cartilage morphology as well as the cartilage biochemical composition [23,24]. Wang et al. demonstrated that regional damage of both femorotibial cartilage and menisci was associated with the severity of OA using T1ρ MRI mapping in subjects with various stages of OA [23]. They reported that the T1ρ values of femoral anterior cartilage sub-region and the medial posterior sub-region of the meniscus are both higher in moderate-severe OA than those in doubtful-mild OA. However, it was unclear whether symptomatic meniscal tear was associated with occult cartilage damage in patients without OA. Kai et al. established an association between meniscal signal-complex tears and increased T2 values in tibial articular cartilage [24]. They reported that T2 values of femoral condyle cartilage was not associated with meniscal tear. The differences between T1ρ and T2 in the sensitivity and diagnostic power for the detection of early macromolecular changes in cartilage might have caused the discrepancy between their results and ours. Furthermore, research into the investigation of the heterogeneity of cartilage T1ρ values in relation to joint morphology is still new. This study suggests that a heterogeneous distribution of cartilage denaturisation associated with meniscal degenerative tears is a precursor of future OA progression.

Additionally, the facts obtained in this study suggest a potential risk for progression of cartilage damage after a meniscal surgery for these patients despite no radiographic OA being observed. Clinical application of this MRI technique as a routine evaluation for meniscal tear could be a help for surgeons. A longitudinal study should be able to reveal whether preoperative evaluation of cartilage macromolecular quality using this MRI technique can predict postoperative progression of cartilage damage.

Conclusions

This study suggested that degeneration of the cartilage matrix had already occurred in patients with degenerative meniscal tears although morphological changes of the articular cartilage had not been detected. This change appeared in the superficial zone of the articular

cartilage at the point where femoral and tibial cartilage contact each other during slight flexion. Our results suggest that this area is the origin of early biochemical changes leading to OA progression associated with degenerative meniscal tears.

Abbreviations

OA: Osteoarthritis; MRI: Magnetic resonance imaging; PG: Proteoglycan; KL: Kellgren-Lawrence; ICC: Interclass correlation coefficient; ROI: Region of interests.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

HM carried out the image analysis of MRI, participated in the data management and drafted the manuscript. KO (Ken Okazaki) conceived of the study, and participated in its design and coordination and helped to draft the manuscript. YT carried out the reconstruction of T1ρ Mapping MRI and helped to draft the manuscript. KO (Kanji Osaki) participated in the measure of T1ρ values and the statistical analysis and helped to draft the manuscript. YM and HH participated in the clinical examination including MRI and radiography and helped to draft the manuscript. YI involved in revising the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

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Subclinical cartilage degeneration in young athletes with posterior cruciate ligament injuries detected with T1ρ magnetic resonance imaging mapping

Ken Okazaki · Yukihisa Takayama · Kanji Osaki · Yoshio Matsuo · Hideki Mizu-uchi · Satoshi Hamai · Hiroshi Honda · Yukihide Iwamoto

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Abstract
Purpose Prediction of the risk of osteoarthritis in asymptomatic active patients with an isolated injury of the posterior cruciate ligament (PCL) is difficult. T1ρ magnetic resonance imaging (MRI) enables the quantification of the proteoglycan content in the articular cartilage. The purpose of this study was to evaluate subclinical cartilage degeneration in asymptomatic young athletes with chronic PCL deficiency using T1ρ MRI.
Methods Six athletes with chronic PCL deficiency (median age 17, range 14–36 years) and six subjects without any history of knee injury (median age 31.5, range 24–33 years) were recruited. Regions of interest were placed on the articular cartilage of the tibia and the distal and posterior areas of the femoral condyle, and T1ρ values were calculated.
Results On stress radiographs, the mean side-to-side difference in posterior laxity was 9.8 mm. The T1ρ values at the posterior area of the lateral femoral condyle and the superficial layer of the distal area of the medial and lateral femoral condyle of the patients were significantly increased compared with those of the normal controls ($p < 0.05$). At the tibial plateau, the T1ρ values in both the medial and lateral compartments were significantly higher in patients compared with those in the normal controls ($p < 0.05$).
Conclusion T1ρ MRI detected unexpected cartilage degeneration in the well-functioning PCL-deficient knees of young athletes. One should be alert to the possibility of subclinical cartilage degeneration even in asymptomatic patients who show no degenerative changes on plain radiographs or conventional MRI.
Level of evidence IV.

Keywords Posterior cruciate ligament (PCL) · Conservative treatment · Osteoarthritis · Cartilage · Magnetic resonance imaging (MRI)

Introduction

The best treatment strategy for posterior cruciate ligament (PCL) injury and the operative indications remains controversial [3, 6, 15]. Non-operative treatment is generally recommended for isolated grade I and II PCL injuries. Treatment of isolated grade III PCL injuries is somewhat controversial. In general, operative treatment is recommended for chronic grade III PCL injuries in relatively young and active patients who become symptomatic because of pain or instability despite an appropriate rehabilitation program [3, 24]. However, non-operative treatment may be chosen even for young and active patients with grade III injuries if the patient remains complaint-free after initial rehabilitation [7]. A number of clinical studies suggest that the symptoms are not correlated with the grade of instability in isolated PCL injuries [7, 17, 19, 20].

Another concern in the treatment of PCL injury is the degenerative changes in articular cartilage during long-term follow-up. Although favourable subjective results

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of non-operative treatment on most isolated PCL injuries are reported to last up to 10 years, several studies suggest that articular cartilage deteriorates over time [2, 10, 17, 19, 20]. This also occurs in patients treated surgically [11, 16, 18, 24]. Most clinical studies utilise plain weight-bearing radiographs to assess the degenerative changes in articular cartilage [1]. These clinical studies have yielded little information on early degenerative changes in the articular cartilage matrix molecules. Furthermore, it is unclear what part of the articular cartilage of the knee joint would bear the brunt of the degenerative stress caused by kinematic loads in young athletes with PCL deficiency.

T1ρ magnetic resonance imaging (MRI) mapping enables the assessment of early degenerative changes in articular cartilage by detecting the proteoglycan content of the cartilage matrix [4, 8]. The clinical relevance of T1ρ MRI mapping has been reported in several in vivo studies [13, 14]. In this study, T1ρ MRI mapping was performed on six young athletes with chronic PCL deficiency. Although this is a preliminary study with a small sample size, the results showed unexpected cartilage damage in the asymptomatic knees of young athletes, suggesting a risk of cartilage deterioration in highly active patients with high instability.

Materials and methods

Six athletes with chronic PCL deficiency (median age 17, range 14–36 years) and six healthy volunteers (median age 31.5, range 24–33 years) without any knee problems were recruited for this study. The PCL deficiency was diagnosed using physical examinations, stress radiographs, and MRI findings. All patients participated in competitive team sports (soccer, baseball, rugby, and Japanese archery; the latter requires repetitive kneeling and squatting). All the patients had an isolated PCL injury without injury to any other ligaments including the posterolateral corner structures. All the patients were initially treated non-surgically with bracing for several months after the injury and given physical therapy for range of motion and muscle exercises. After the non-surgical treatment, they returned to their sports activities. One patient, who was injured 6 years ago, played on a competitive baseball team for 3 years and took part in Japanese archery for 3 years. Five patients had no complaints during sports activities, and one of them complained of occasional weakness and slight pain during sports activities. The median duration after PCL injury was 17 months (range 6–216 months). The mean Lysholm score was 98.3, and the mean Tegner score was 7.2.

Five of the healthy volunteers had participated in competitive or recreational sports (soccer, basketball, and baseball). The mean Tegner score of all the healthy volunteers was 6.3.

The study protocol was approved by the Institutional Ethics Board of Kyushu University (approval number: 23–75) and was carried out in accordance with the Tenets of the Declaration of Helsinki.

MRI scanning

The subjects underwent MRI of the knee, which was performed using a 3-Tesla MR system (Achieva 3.0T, Quasar Dual, Philips Healthcare, Best, The Netherlands) equipped with an 8-channel phased-array coil. The imaging protocol included a sagittal fat-suppression turbo spin echo T2-weighted imaging (FS-T2WI) sequence with the following parameters: repetition time (TR), 4,675 ms; echo time (TE), 71 ms; flip angle, 90°; turbo spin echo factor, 16; field of view (FOV), 140 × 140 mm; matrix, 400 × 400; slice thickness, 3 mm; slice gap, 0 mm; number of slices, 26; and number of excitations, 1. FS-T2WI was used as an anatomical reference as well as for diagnosis of injury to joint structures including ligaments, menisci, and cartilage.

Sagittal two-dimensional T1ρ mapping was performed without parallel imaging and each sequence used the following parameters: TR, 4.7 ms; TE, 2.4 ms; flip angle, 35°; FOV, 140 × 140 mm; matrix, 320 × 320; slice thickness, 3 mm; slice gap, 0 mm; number of slices, 26; NEX, 1; spin-lock pulse frequency, 500 Hz; and time of spin-lock (TSL), 1, 20, 40, 60, and 80 ms. Although flip angle was low, it did not affect T1ρ contrast because the shot interval was 6,000 ms between each slice acquisition and the k-space was filled using low–high ordering. T1ρ mapping was used for quantitative assessment.

Assessment of the T1ρ maps

T1ρ maps were estimated using pixel-by-pixel fitting of signals obtained from five different T1ρ-prepared images acquired with five different TSLs (1, 20, 40, 60, and 80 ms) and using the following exponential T1ρ decay equation: $S(TSL) \alpha \exp(-TSL/T1\rho)$, where S(TSL) is the signal intensity in the T1ρ-prepared image with a given TSL. We produced T1ρ maps using Philips Research Integrated Development Environment (PRIDE) software written in Interactive Data Language (IDL 6.3, ITT Inc., Boulder, CO, USA).

For the evaluation of T1ρ maps, regions of interest (ROIs) were drawn corresponding to parts of the full thickness of cartilage on the FS-T2WI, which was used after its in-plane resolution was adjusted to that of the T1ρ map. We picked up four adjacent sagittal slices at the centre of the medial and lateral compartment and evaluated the femoral condyle and tibial plateau in each slice. In the femoral condyle cartilage, ROIs were drawn around the distal and posterior areas. The distal area extends from the boundary

adjacent to the anterior edge of the anterior meniscal body to the centre of the posterior meniscal body. The posterior area extends from the posterior edge of the distal area to an edge that is 90° posterior with respect to the femoral shaft axis. In the tibial plateau cartilage, ROIs were drawn to cover the entire cartilage area. ROIs were then divided into superficial and deep zones of equal thickness (Fig. 1). ROIs in the same locations were drawn on the T1ρ maps, and mean T1ρ values and standard deviations (SD) were calculated for each slice. The mean T1ρ values and SD of the ROIs of similar areas were calculated again from four slices. These analyses were performed by an experienced radiologist using "medical image processing, analysis, and visualisation" software (MIPAV, Biomedical Imaging Research Services Section, Center for Information Technology, National Institutes of Health, Bethesda, MD, USA). All measurements were performed by one observer (K. Okazaki) and were repeated in a blinded manner during the course of two sessions 1 month apart. Another observer (K. Osaki) independently made measurements of five randomly selected knees.

Stress radiography

A stress radiography examination was performed by applying a 15-kg posterior load to the knee in 90° of flexion using a Telos arthrometer (Telos, Weiterstadt, Germany). Side-to-side differences in posterior translation of the tibia were recorded. A weight-bearing anterior-posterior radiograph was also taken for the knee in 30° of flexion.

Statistical analyses

For comparison of the T1ρ values between the PCL-deficient patients and the normal subjects, nonparametric comparisons were performed using a Mann–Whitney *U* test. *p* values of <0.05 were considered significant.

Results

All the PCL-deficient patients showed posterior laxity with an average of 9.8 mm (range 5.1–13.5 mm) in the stress radiographs. No patients showed osteoarthritic changes including joint space narrowing on weight-bearing anterior-posterior radiographs of the knees in 30° of flexion. These patients had no or mild symptoms during sports activities. The fat-suppression turbo spin echo T2-weighted images showed no severe degenerative changes in their cartilage including thinning, injury, or erosion (Fig. 2a, b). However, the T1ρ mapping MRI revealed an increase in T1ρ values in the articular cartilage of the femoral condyle and tibial plateau in both the medial and lateral compartments (Fig. 2c, d; Table 1). The differences in T1ρ values were significant in the tibial plateau in both the medial and lateral compartments ($p < 0.05$). The T1ρ values of the distal area of the femoral condyle (especially the superficial layer) and the posterior area of the lateral femoral condyle were also significantly higher in the patients. Relatively wide variations were also observed in the T1ρ values among patients. A very active soccer player (Tegner score = 9) showed a very high increase in T1ρ values in all areas, while relatively mild increases in T1ρ values were observed in the less active patients (i.e. Tegner score = 5) or the patients with a relatively recent injury. In contrast, although one normal volunteer was a very active athlete (Tegner score = 9), the T1ρ values were not high compared with others (Fig. 2e, f). Intraobserver reliability of the measurements was 0.89. Interobserver reliability of the measurements was 0.81.

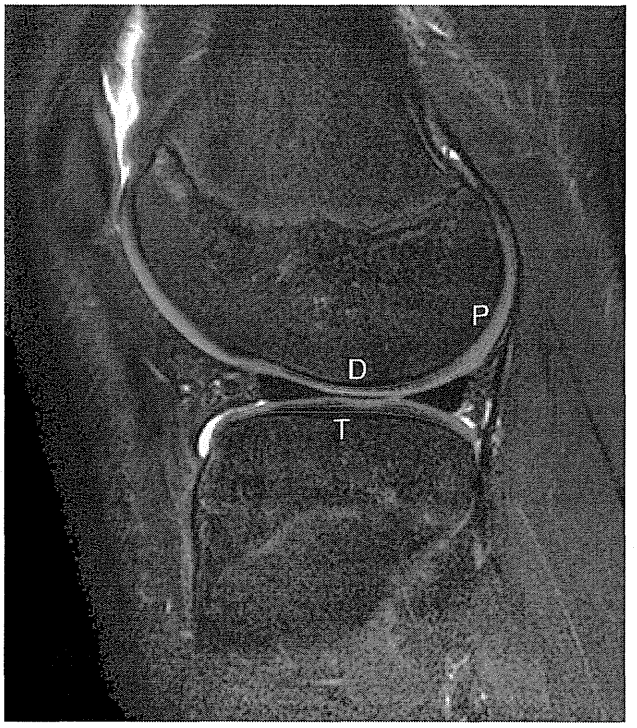

Fig. 1 Regions of interest (ROIs) were drawn on the articular cartilage in fat-suppression turbo spin echo T2-weighted imaging (FS-T2WI) of the knee. ROIs were in the distal area (*D*) and posterior area (*P*) of the femoral condyle and in the tibial plateau (*T*). ROIs were then divided into superficial and deep layers. The image of the lateral compartment in a case with PCL deficiency is shown

Discussion

The most important finding of this study was that degeneration of articular cartilage can occur in an asymptomatic PCL-deficient knee especially when the patient is a very

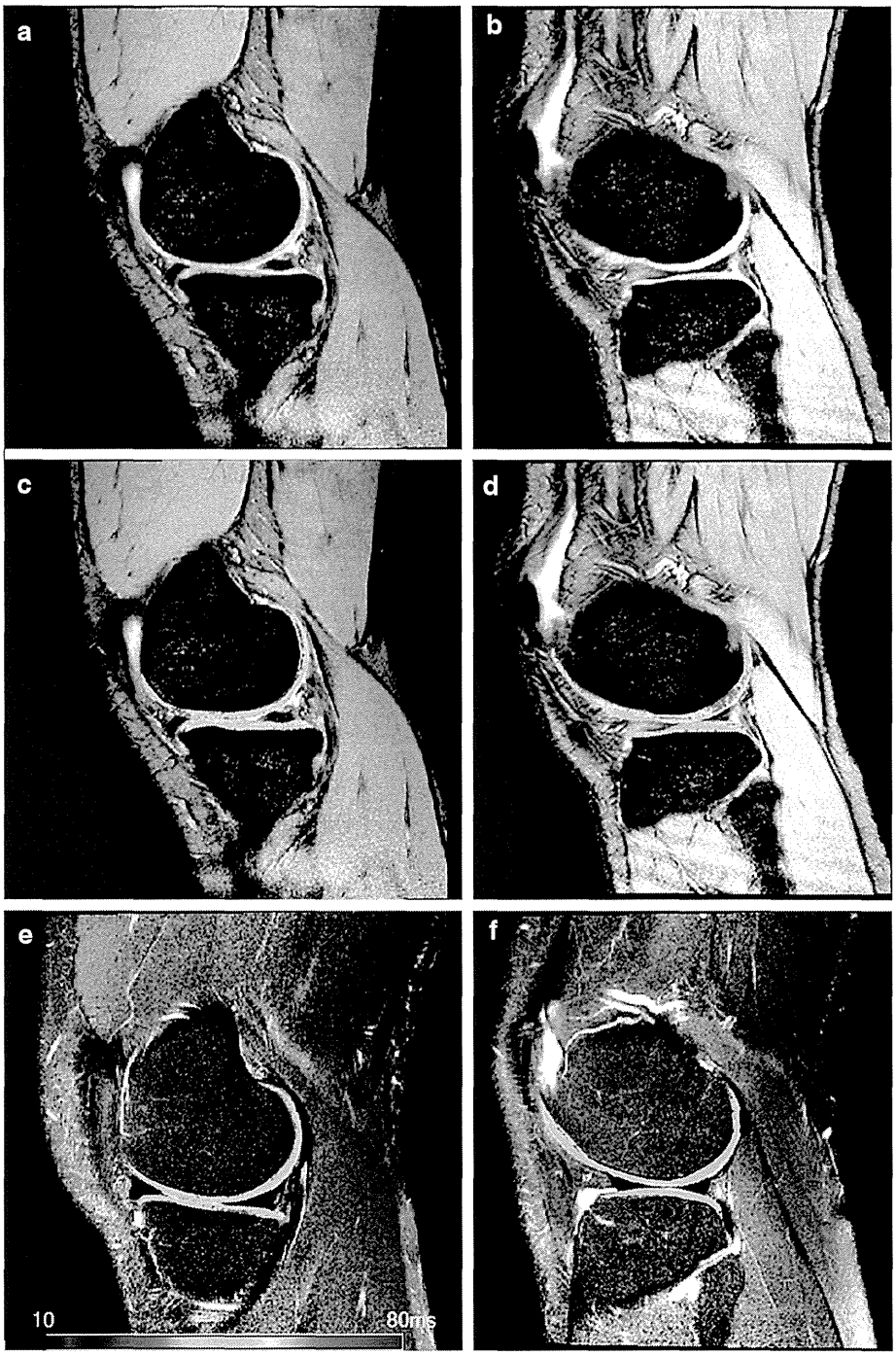

Fig. 2 Fat-suppression turbo spin echo T2-weighted imaging (FS-T2WI) of the medial compartment (**a**) and lateral compartment (**b**) in a case with PCL deficiency. **c**, **d** T1ρ maps of images corresponding to **a** and **b**, respectively. T1ρ maps of the medial compartment (**e**) and lateral compartment (**f**) in a normal control subject. The *colour map* indicates the T1ρ value

active athlete and he/she has a significant instability of the knee. T1ρ MRI mapping enables us to evaluate the present integrity of cartilage matrix in vivo and may contribute towards research to predict the risk of osteoarthritis (OA).

Several MRI techniques have been developed to determine changes in the biochemical composition and macromolecular structure of hyaline cartilage. T1ρ mapping and delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) techniques can be used to evaluate the proteoglycan content and distribution, while T2 mapping can be used to evaluate the extent of collagen fibre disorganisation by detecting the interaction between water and collagen molecules [4, 8]. Because T1ρ and T2 mappings require no injection of contrast agents, their potential for use in the detection of early degenerative changes of articular cartilage in OA has attracted attention. A comparative study of T1ρ and T2 mappings revealed that both techniques could detect cartilage degeneration in early OA using relaxation