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Natural evolution of popliteomeniscal fascicle tears over 2 years and its association with lateral articular knee cartilage degeneration in patients with traumatic anterior cruciate ligament tear

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Abstract
Objectives To assess (i) normal imaging anatomy of the popliteomeniscal fascicles, (ii) prevalence and natural evolution of popliteomeniscal fascicle tears (PMFT) in subjects with traumatic anterior cruciate ligament (ACL) tears over 2 years and (iii) compare knee cartilage degeneration in subjects with and without PMFT longitudinally.

Methods 57 subjects with ACL tears were screened for PMFT. Morphological (high-resolution 3D fast spin-echo) and compositional (T1ρ and T2 mapping) MR imaging was performed prior to and 2 years after ACL reconstruction. Differences of morphological and compositional parameters were compared between subjects with and without PMFT using logistic regression, adjusting for age, sex and BMI.

Results In 24% (n = 14) of the subjects with ACL tear a PMFT was detected on baseline MRI. One subject with PMFT developed a meniscal tear over 2 years. Cartilage $\Delta T1\rho$ of the lateral femur increased significantly more in subjects with isolated PMFT compared to controls (mean difference, 2.0 ± 2.9 vs. −1.3 ± 1.6, $p$ = 0.027).

Conclusion PMFT detected by MRI are a common finding in subjects with ACL tears. Subjects with these defects showed higher compositional cartilage deterioration compared to controls, over 2 years in the lateral femoral compartment, indicating accelerated cartilage degeneration.

Key Points
- Popliteomeniscal fascicle lesions are a common finding in subjects with ACL tears.
- Progression to a meniscal tear over 2 years is not frequent.
- Anteroinferior popliteomeniscal fascicle is injured most frequently.
- Patients with popliteomeniscal fascicle lesions showed accelerated cartilage degeneration.

Keywords Magnetic resonance imaging · Cartilage · Anterior cruciate ligament injuries · Meniscus · Popliteomeniscal fascicle

Abbreviations
ACL · Anterior Cruciate Ligament
BMI · Body Mass Index
CUBE · High-Resolution 3D Fast Spin-echo
FSE · Fast Spin-echo
ICC · Intraclass Correlation Coefficient
MRI · Magnetic Resonance Imaging
PMF · Popliteomeniscal Fascicle
PMFT · Popliteomeniscal Fascicle Tears
Introduction

The popliteomeniscal fascicles (PMF) are posterolateral meniscocapsular extensions that blend inferiorly into the popliteus musculotendinous region and allow the tendon to pass from an intra-articular to an extra-articular compartment while maintaining the compartmental integrity of the knee joint [1, 2].

The PMF are composed of three distinct fasciculi, the anteroinferior, posterosuperior and posteroinferior fasciculi, which are attached to the lateral meniscus at the popliteal hiatus [3]. The PMF are considered functionally important stabilizers of the lateral meniscus, working in conjunction with the popliteus musculotendinous unit to prevent excessive lateral meniscal movement and possible entrapment [1, 2].

In an experimental biomechanical study it has been shown that lateral meniscal motion doubled when the PMF were resected in human cadaveric knees [4] and also, tears of the PMF lead to loss of normal peripheral hoop stresses, allowing the lateral meniscus to displace medially [5].

Injuries are often seen in younger athletes engaging in sports that involve repetitive twisting. Mechanisms of injury can involve a single traumatic event or an insidious onset after repeated microtrauma [4, 6].

Tears of PMF are commonly under-recognized both clinically and on imaging studies and are reported [7, 8] to occur in association with acute anterior cruciate ligament tears in 25% of these patients. Isolated tears of the PMF can be symptomatic and manifest as localized posterolateral pain and locking of the knee joint [1, 2, 9].

Without proper diagnosis and treatment, popliteomeniscal fascicle tear (PMFT) can lead to continued instability of the lateral meniscus increasing the risk of developing complex meniscal tear and articular cartilage damage [10].

To date, little is known about the natural evolution of these lesions and we hypothesized that this type of meniscal pathology is related to the development of meniscal tear and accelerated cartilage degeneration longitudinally.

The purpose of our study was therefore (i) to examine the normal anatomy of the popliteomeniscal fascicles with specific reference to the number of popliteomeniscal fascicles in a non-pathologic knee using 3-T high resolution 3D-fast spin-echo (FSE) sequences, (ii) to investigate the frequency and the natural evolution of the popliteomeniscal fascicle tears longitudinally in subjects with ACL tear and (iii) to compare lateral compartment cartilage degeneration over 2 years using quantitative compositional MR imaging techniques (T1p and T2 relaxation time mapping) between subjects with isolated popliteomeniscal fascicle tears and subjects without any meniscal and popliteomeniscal pathology.

Material and methods

Subjects

For this study, 57 subjects with complete traumatic acute ACL tear (32 women; age 32.6 ± 8.3 years; BMI 24.5 ± 3.5 mg/m²) from an ongoing prospective study were screened for popliteomeniscal fascicle tears. All patients gave written informed consent and the study was approved by our institutional review board. Per study protocol, we included subjects with clinically diagnosed acute complete ACL rupture, confirmed by preoperative MRI, that underwent ACL reconstruction as well as standard pre- and postoperative rehabilitation. Complete preoperative MRI and clinical examination were a priori inclusion criteria as well as arthroscopic surgery performed within 12 weeks after injury. Exclusion criteria were history of osteoarthritis, inflammatory arthritis, previous injury and surgery and repeated injuries of the knee during the follow-up period. All subjects underwent knee MRI imaging 2 years after surgery.

In this population, 14 subjects were identified that showed popliteomeniscal fascicle tears, according to the imaging criteria described below. Of the remaining 43 subjects, 33 subjects were excluded from this analysis because of the presence of a meniscal tear at baseline. Ten subjects without any meniscal abnormality at baseline and follow-up were identified and used as controls for the comparison of the development of cartilage composition over 2 years. The MRI of the contralateral knees of these subjects (n = 57) at baseline was assessed to study the contralateral PMF as a standard of reference.

In order to investigate the effect of isolated popliteomeniscal fascicle tears on cartilage in subjects with ACL tear, we excluded subjects with associated lateral meniscal tear at baseline (n = 3) and those who developed a meniscal tear at follow-up (n = 1). The final study population consisted of 10 subjects with ACL tear and isolated popliteomeniscal fascicle tear (cases) and 10 subjects with ACL tear without any meniscal abnormality (control group). All these subjects underwent ACL reconstruction without a meniscal repair. The subject selection process is shown in Fig. 1.

Arthroscopy surgery

All 57 subjects underwent arthroscopic surgery and ACL reconstruction with the standard-of-care anatomic reconstruction of the ACL performed after injury and the menisci pathology was confirmed by arthroscopy in all cases.

Imaging protocol and analysis

Bilateral knee MRI examinations were acquired using a 3-T MRI scanner (General Electric Healthcare, Milwaukee, WI)
with an 8-channel knee coil (Invivo Inc, Gainesville, FL). The imaging protocol included a high-resolution 3D fast spin-echo (CUBE) sequence and quantitative compositional \( T_1 \rho \) and \( T_2 \) relaxation time mapping. The detailed MR acquisition parameters (morphological and compositional) are summarized in Table 1. Preoperative and 2-year follow-up MRIs were available for all subjects and obtained with identical sequence parameters.

**Morphologic imaging**

In order to analyse the normal 3.0-T MR imaging anatomy (Fig. 2) of the PMF we used images of the contralateral knee. All 57 subjects showed no abnormalities of the menisci and cartilage or effusion. Two radiologists evaluated all images independently and they were blinded to clinical information and time point, and the cases of disagreement were reconciled by consensus. Visibility or non-visibility was documented for the anteroinferior, posterosuperior and posteroinferior fascicles in all 57 subjects, using the sagittal 3D FSE sequence. The anteroinferior popliteomeniscal fascicle was defined as present if it originated from the lateral surface of the body of the lateral meniscus and formed the floor and lateral wall of the hiatus popliteus. The posterosuperior popliteomeniscal fascicle was identified as present if it originated from the superior edge of the posterior horn of the lateral meniscus and formed the roof and medial wall of the hiatus and the posteroinferior popliteomeniscal fascicle was identified medial to the popliteal hiatus as a medial aponeurotic extension from the musculotendinous junction of the popliteus muscle, which attached to the inferior edge of the posterior horn of the lateral meniscus, immediately beneath the origin of the meniscofemoral ligament of Wrisberg [11, 12].

As the posterosuperior and the anteroinferior fascicles were identified (as detailed below) in all contralateral knee (“non-pathologic knees”) a popliteomeniscal fascicle tear was defined if this structure was disrupted or not identified in more than two consecutive images (Figs. 3, 4). As the posteroinferior fascicle was not identified in all subjects on the contralateral (healthy) side it was excluded from this specific analysis, and only the posterosuperior and anteroinferior were taken into account. All 57 post-traumatic knee

| Table 1 Morphologic and compositional sequences parameters |
|---------------------------------|--------|--------|--------|--------|---------|-----------------|-----------------|
| Sagittal Fat Sat 3D FSE | 1500  | 23     | 50     | 32     | 2       | 16   | 384 × 384 | Fat Sat         |
| Sagittal PD          | 5100  | 30     | 62.5   | 13     | 1–2     | 16   | 512 × 480 | TRF             |
| Coronal PD          | 5100  | 30     | 62.50  | 13     | 1       | 14   | 512 × 416 | TRF             |
| 3D T1ρ             | 9.3   | 3.7    | –      | –      | –       | 14   | 256 × 192 | 4 mm            |
| 3D T2              | 9.3   | 3.7    | –      | –      | –       | 14   | 256 × 192 | Preparation TE (ms): 2.9, 13.6, 24.3, 45.6 |

\( TR \) repetition time, \( TE \) echo time, \( BW \) bandwidth, \( ETL \) echo train length, \( NEX \) number of excitations, \( FOV \) field of view, \( Add opt \) additional option, \( Fat Sat \) fat saturation, \( PD \) proton density, \( TRF \) tailored radio frequency pulse, \( TSL \) time of spin lock, \( FSL \) frequency of spin lock
Preoperative MR examinations were independently evaluated by two radiologists for the presence of a tear of the posterosuperior or anteroinferior fascicles.

Follow-up scans after 2 years were assessed for the presence/development of meniscal tears or any change of meniscal morphology on the longitudinal analyses. Meniscal abnormalities were classified as a meniscal tear if they communicated with the superior, inferior or free edge of the meniscal surface on at least two consecutive images or two different planes [13]. Radiologists were blinded to preoperative findings and MRIs of each follow-up time point were read at least 1 month after the baseline MRI readings or after the previous reading had been performed.

**T1 and T2 relaxation time mapping**

Cartilage was segmented semi-automatically on sagittal 3D FSE images by using a Matlab-based algorithm developed at our institution, as previously described [14]. The lateral femoral condyle and tibia cartilage compartments were analysed at baseline and 2 years after surgery. Piecewise rigid registration was applied along both T1ρ and T2 echoes to account for

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**Fig. 2** Sagittal 3D fast spin-echo sequence of a contralateral knee of a 28-year-old woman showing the normal MR imaging of the popliteomeniscal fascicles. **a** The posterosuperior popliteomeniscal fascicle (arrow) is identified originating from the superior edge of the posterior horn of the lateral meniscus and forming the roof and medial wall of the popliteus hiatus. The anteroinferior popliteomeniscal fascicle (arrow head) is identified originating from the lateral surface of the body of the lateral meniscus and forming the floor and lateral wall of the hiatus popliteus. **b** The posteroinferior popliteomeniscal fascicle (straight arrow) is identified medial to the popliteal hiatus as a medial aponeurotic extension from the musculotendinous junction of the popliteus muscle, which attaches to the inferior edge of the posterior horn of the lateral meniscus, immediately beneath the origin of the meniscofemoral ligament of Wrisberg (curved arrows).

**Fig. 3** Sagittal 3D fast spin-echo sequence before surgery of a 23-year-old man with ACL tear. **a** Preoperative MRI with a tear of the posterosuperior and anteroinferior popliteomeniscal fascicles (arrows). We also identified a bone marrow oedema pattern (*) at the central lateral condyle, a typical MRI finding in subjects with ACL tear. **b** The contralateral, control knee of the same subject is shown with normal anatomy of the posterosuperior and anteroinferior popliteomeniscal fascicles (arrows).
non-rigid movement of the lateral femur and tibia. T1ρ and T2 maps were reconstructed by fitting the echo images pixel by pixel to the following equations: \( S(TSL) \propto S_0 \exp\left(-\frac{TSL}{T1\rho}\right) \) for T1ρ and \( S(TE) \propto S_0 \exp\left(-\frac{TE}{T2}\right) \) for T2, where TSL is the time of spin lock, TE is the echo time and S is the signal intensity. 3D FSE images were registered to T1ρ and T2 maps, and cartilage contours generated from the 3D FSE images were overlaid onto the T1ρ and T2 maps. T1ρ and T2 maps at follow-up time points were also registered to baseline. Mean T1ρ and T2 values were calculated for each defined compartment at both time points. Changes in T1ρ and T2 values from baseline to 2 years were defined as \( \Delta T1\rho = T1\rho_{2\text{-year}} - T1\rho_{\text{baseline}} \); \( \Delta T2 = T2_{2\text{-year}} - T2_{\text{baseline}} \).

Statistical analysis

Statistical analysis was performed with SPSS (Version 22.0, Armonk, NY: IBM Corp.) using a two-sided 0.05 level of significance. Independent sample t tests (for numerical and approximately normally distributed data) and Pearson’s chi-square tests (for categorical variables) were used to evaluate differences between subjects with and without popliteomeniscal fascicle tear.

Differences of T1ρ and T2 at baseline and 2 years and \( \Delta T1\rho \) and \( \Delta T2 \) measurements subscale scores were compared between subjects with and without popliteomeniscal fascicle tear using logistic regression, adjusting for age, sex and BMI (body mass index).

Reproducibility

Inter-reader reproducibility for the evaluation of the presence of popliteomeniscal fascicle lesion was assessed between the two radiologists in all 57 cases by using the intraclass correlation coefficient (ICC), which was 0.87 (95% confidence interval, 0.82–0.92). For intrareader reproducibility analysis, both readers repeated the readings in 25 randomly selected patients after at least 2 weeks. The ICC for intrareader reproducibility was 0.91 (95% confidence interval, 0.88–0.93).

Results

Analysis of the contralateral, reference knees

The anteroinferior and posterosuperior fascicles were identified in all contralateral knees of the subjects. The posteroiinferior fascicle was identified in only 45% (26/57) subjects.

Prevalence of popliteomeniscal fascicle tears, baseline imaging characteristics and evolution over 24 months

In subjects with ACL tears (n = 57), any type of PMFT was detected in 24% (n = 14) of the cases. Three (21%) of these 14 subjects had a PMFT associated with a tear of the posterior horn of the lateral meniscus (all of them vertical tears), while the remaining 11 subjects (79%) had an isolated PMFT at baseline. One subject (1/11) with an isolated PMFT developed a meniscal tear (horizontal-oblique) in the posterior horn of the lateral meniscus over 2 years. In all subjects with both meniscal tears and PMFT, both fascicles (posterosuperior and anteroinferior) were always injured.

In subjects with an isolated PMFT (11 subjects), the anteroinferior fascicle was torn in all cases (100%), 45% of subjects (5/11) presented with lesions of both fascicles and 55% of subjects (6/11) with just an anteroinferior lesion (but in all cases the posterosuperior fascicle presented with signal abnormality). Imaging characteristics of the PMFT are shown in Fig. 5.

Compositional analysis of cartilage T1ρ and T2 at baseline and after 24 months

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The $\Delta T_1 \rho$ from baseline to 2 years increased significantly more in subjects with isolated PMFT than in controls in the lateral femur ($2.0 \pm 2.9$ ms vs. $-1.3 \pm 1.6$ ms, $p = 0.027$); and a statistical trend was found in the lateral tibia ($4.6 \pm 2.3$ ms vs. $2.4 \pm 1.9$ ms, $p = 0.083$), suggesting accelerated progression of cartilage degeneration in the lateral compartment over 2 years, when compared to ACL injured patients without meniscal pathology. The $\Delta T_2$ over 2 years did not show significant differences ($p > 0.05$) between both groups.

The absolute $T_1 \rho$ and $T_2$ values at baseline and 2 years and $\Delta T_1 \rho$ and $T_2$ values of subjects with and without PMFT at the lateral femur and lateral tibia are summarized in Tables 2 and 3.

**Discussion**

Our study provides pertinent insights into the anatomy and ACL tear-related pathology of the popliteomeniscal fascicles: it showed that the posterosuperior and anteroinferior popliteomeniscal fascicles were consistently identified with 3D FSE imaging while the posteroinferior fascicle was only identified in 45%. It also demonstrated that popliteomeniscal fascicle tears were found in approximately 25% of patients after acute ACL tear. They were associated with a lateral meniscal tear in about 21% at baseline, while the vast majority of the subjects did not develop an incident meniscal tear over 2 years. Moreover, our study demonstrated that subjects with an ACL tear and an isolated popliteomeniscal fascicle tear undergo accelerated cartilage degeneration in the lateral femur over 2 years compared to controls without popliteomeniscal fascicle tears.

We found that the anteroinferior and posterosuperior popliteomeniscal fascicles were seen on all 3.0-T volumetric images, while the posteroinferior popliteomeniscal fascicle was only detected in 45% of the non-injured (contralateral knees) in our study. Similar findings were reported by

**Table 2** Summary of $T_1 \rho$ values at baseline and 2-year follow-up and $\Delta T_1 \rho$ 2 years after anterior cruciate ligament surgery

<table>
<thead>
<tr>
<th>$T_1 \rho$ values at BL and 2 years*</th>
<th>$\Delta T_1 \rho$ 2 years*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fascicle lesion</td>
<td>Controls</td>
</tr>
<tr>
<td>Global LT</td>
<td>$37.3 \pm 2.3$</td>
</tr>
<tr>
<td>Global LF</td>
<td>$31.9 \pm 3.0$</td>
</tr>
</tbody>
</table>

$LT$ lateral tibia, $LF$ lateral femur

*Data expressed as mean ± standard deviation
Peduto et al. [12] using 1.5-T MR arthrographic studies in cadavers regarding the anteroinferior and posterosuperior popliteomeniscal fascicles (100%) and in our study we identified the posteroinferior fascicle slightly more often (45% vs. 40%), probably because we used 3.0-Tesla high-resolution 3D FSE sequences.

The anteroinferior fascicle was most frequently injured, and every subject with a posterosuperior fascicle tear also presented an anteroinferior fascicle tear. Staubli and Birrer [7] showed that 25% of patients with acute ACL tear that underwent knee arthroscopy had a lesion of the anteroinferior popliteomeniscal fascicle and 7.5% had lesions of both the posterosuperior and anteroinferior popliteomeniscal fascicles, findings which are similar to our study.

It is still not well established in the literature how frequently lateral meniscal tears and popliteomeniscal fascicle tears in subjects with acute ACL tear are associated, and it is also not known how frequently lateral meniscal tears develop in subjects with isolated popliteomeniscal fascicle lesion. Our study showed that these lesions are present combined in 21% of patients at baseline. In all cases both fascicles (posterosuperior and anteroinferior) were injured. Therefore, whenever there is a lesion of the posterosuperior fascicle an association with lateral meniscal tear should be suspected. Interestingly, De Smet et al. [15] showed that the presence of superior popliteomeniscal fascicle abnormalities is significantly associated with a tear of the lateral meniscus.

An in vitro, biomechanical study by Simonian et al. [1] demonstrated that lateral meniscal motion and thus instability doubled when the popliteomeniscal fascicles were resected in cadaveric knees, and the authors hypothesized that this could lead to an increased number of lateral meniscal lesions, but in our cohort just one patient (1/11) developed lateral meniscal tear over 2 years.

In order to study early cartilage degeneration we used advanced quantitative MRI techniques (T1ρ and T2 relaxation time mapping) to demonstrate biochemical changes of the cartilage matrix, even before morphologic cartilage lesion occur.

Several previous studies have evaluated cartilage T1ρ and T2 values in order to detect compositional cartilage matrix changes after ACL injury and reconstruction [14, 16, 17]. Therefore, these techniques are adequate to analyse initial cartilage damage in the post-traumatic knee.

The T1ρ and T2 absolute values at the lateral tibia and lateral femur tended to be higher at baseline in the controls, probably because they were significantly older and had higher BMI. Interestingly, after 2 years the T1ρ and T2 absolute values of the subjects with isolated popliteomeniscal fascicle tear increased and presented higher absolute values in T1ρ and T2 at the lateral tibia and lateral femur. Most interestingly, our study demonstrated that after ACL injury, subjects with an isolated popliteomeniscal fascicle tear showed accelerated progression of cartilage degeneration in the lateral femur over 2 years, even if they were younger than the control group. Only T1ρ showed significance and the T2 values showed a similar trend but no significance, probably because T1ρ values have higher sensitivity to detect compositional cartilage matrix changes after ACL injury [14, 16, 17].

Similar to our study, Shin et al. [10] found osteochondral lesions in the posterior area of the lateral femoral condyle during arthroscopic evaluation of subjects with popliteomeniscal fascicle tears. They believed that the cause of osteochondral lesions was the repetitive entrapment of the lateral meniscus caused by posterolateral translation in a situation where a popliteomeniscal fascicle tear occurs. They suggested that if osteochondral lesions develop in the posterior area of the lateral condyle, a popliteomeniscal fascicle tear can be strongly suspected, although it could not be definitively confirmed in all their cases.

The authors believe that the increase of lateral meniscal motion and instability and the repetitive entrapment of the lateral meniscus are the main reasons for accelerated cartilage degeneration in these subjects with popliteomeniscal fascicles lesions.

### Table 3

<table>
<thead>
<tr>
<th>T2 values at BL and 2 years</th>
<th>T2 values at BL</th>
<th>p value</th>
<th>T2 values 2 years</th>
<th>T2 values 2 years</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fascicle lesion</td>
<td>Controls</td>
<td></td>
<td>Fascicle lesion</td>
<td>Controls</td>
</tr>
<tr>
<td>Global LT</td>
<td>29.3 ± 1.6</td>
<td>29.8 ± 2.4</td>
<td>0.292</td>
<td>33.3 ± 2.6</td>
<td>32.0 ± 2.8</td>
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<tr>
<td>Global LF</td>
<td>25.1 ± 1.5</td>
<td>25.4 ± 2.2</td>
<td>0.239</td>
<td>26.8 ± 2.4</td>
<td>25.6 ± 1.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ΔT2 BL 2 years</th>
<th>Fascicle lesion</th>
<th>Controls</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global LT</td>
<td>3.8 ± 2.7</td>
<td>2.5 ± 0.3</td>
<td>0.322</td>
</tr>
<tr>
<td>Global LF</td>
<td>1.7 ± 2.1</td>
<td>0.2 ± 2.0</td>
<td>0.378</td>
</tr>
</tbody>
</table>

LT lateral tibia, LF lateral femur

*Data expressed as mean ± standard deviation
This study has some limitations: firstly, it was based on a relatively small cohort as popliteomeniscal fascicle tears were found in 14 subjects or 25% of subjects with ACL tears, which probably represents the normal prevalence of this finding after ACL tears. It should also be noted that we followed up 24 subjects over 2 years, which is the first longitudinal investigation of this entity.

One of the limitations of our study is the lack of detailed surgical-imaging correlations of popliteomeniscal fascicles lesions in the baseline arthroscopic data, as this specific type of lesion is not fixed routinely in the majority of the ACL tear subjects. However, we could access arthroscopic information about overall meniscal abnormalities (e.g. meniscal tears, meniscal morphology and meniscal mobility) in all subjects.

Subsequently, further subgroup analyses between subjects with isolated anteroinferior fascicle tear and subjects with combined fascicles tears (posteroinferior and anteroinferior) were impossible because of even smaller sample sizes.

In summary, we found that popliteomeniscal fascicles tears detected by MRI are a common finding in subjects with ACL tears. Subjects with these defects showed higher compositional cartilage deterioration compared to patients without meniscal pathology after ACL tear, over 2 years in the lateral femoral compartment, indicating accelerated cartilage degeneration.

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Compliance with ethical standards

Guarantor The scientific guarantor of this publication is Thomas M. Link.

Conflict of interest The authors of this manuscript declare no relationships with any companies whose products or services may be related to the subject matter of the article.

Statistics and biometry No complex statistical methods were necessary for this paper.

Informed consent Written informed consent was obtained from all subjects (patients) in this study.

Ethical approval Institutional review board approval was obtained.

Methodology

• prospective
• observational
• performed at one institution

References