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First detection of primary cilia in injured human anterior cruciate ligament: A pilot study with pathophysiological reflections

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ABSTRACT

The anterior cruciate ligament (ACL) plays a significant role in knee stability, protects the joint under multiple loading conditions and shows complex biomechanics. Beside mechanical stability, the ACL seems to play a crucial role in proprioception, and it is well known, that ACL injuries can cause functional deficits due to decreased proprioception. However, the mechanism of proprioception is not completely understood yet. In this context, primary cilia (PC), which play a significant role in the signaling between the intra- and extracellular space, could be of interest. However, until today, primary cilia are not yet described in human ACL. In total, seven human ACL's underwent transmission electron microscopical examination. Three cadaveric ACL's and four freshly injured ACL's were examined. Single cells of each ACL were examined regarding the presence of axonemes or basal bodies, which represent components of a PC. In total, 276 cells of the cadaveric ACL's and 180 cells of the injured ACL's were examined. Basal bodies could be detected in three of the four specimens of the injured ACL's as well as in one of the three cadaveric ACL's, resulting in a mean positivity of 2.54% in the cadaveric group and 2.78% in the injured group. In case of PC-presence, only one PC per cell could be detected. No statistically significant difference regarding the frequency could be detected between both groups. In this pilot-study, we present for the first time an ultrastructural study of human ACLs with respect to the occurrence of PC and any structural and morphological features of these complex and dynamic cell organelles. PCs are present in almost all non-hematopoietic tissues of the human body. However, there are different reports on the number, incidence, orientation, and morphology of these cell organelles in the respective tissues. Compared to other tissues and ligaments of other species, we found a significantly lower rate of PC positive cells. This observation might represent a tissue-specific characteristic of ACL tissue. However, our observations need to be explored in more detail in further studies.

1. Introduction

The anterior cruciate ligament (ACL) plays a significant role in knee stability and protects the joint under multiple loading conditions. It prevents from anterior tibial translation as well as from internal rotation of the tibia, which indirectly prevents valgus instability [1]. Anatomically, the ACL's origin is the posterior part of the inner wall of the lateral femoral condyle and it approaches on the anteromedial tibial plateau [2]. The ACL consists of two bundles, the anteromedial bundle (AMB) and the posterolateral bundle (PLB). It shows complex biomechanical characteristics with a tightened AMB and a relatively relaxed PLB in flexion and approximately contrary tension-settings in extension [3], which means that different parts within the ligament change their length and tension during motion [2]. The ACL mainly contains of collagen

Abbreviations: ACL, anterior cruciate ligament; PC, primary cilia; AMB, anteromedial bundle; PLB, posterolateral bundle; OA, osteoarthritis; BB, basal body; ECM, extracellular matrix.

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fibers and its vascularization is provided by branches of the middle genicular artery [4]. The ACL belongs to the most common injured structures in the knee, with an incidence of up to 250.000 injuries per year in the United States [5]. In case of ACL-tear, additional injuries of e. g. the menisci or cartilage lesions are common [6]. Due to the high number of injuries, ACL-tears also show a socioeconomic importance, beside its clinical relevance. Clinically, a torn ACL represents a serious injury of the knee and often results in pain, knee instability and increases the risk for the development of an early osteoarthritis (OA). Especially in younger and active patients, ACL-reconstruction is the therapy of choice to restore knee stability. Surgery is performed arthroscopically and the torn ACL gets replaced with autologous tendon-grafts. Most frequently used grafts are the quad-striped semitendinosus-tendon, stripes of the patella- or the quadriceps-tendon with or without an adherent bone block. Grafts can be fixed using several techniques and devices [7]. Despite improved surgical techniques, based on current literature, ACL-reconstruction does not prevent from OA [8].

Beside mechanical stability, the ACL seems to play a crucial role in proprioception, which encompasses sensory information of joint position and joint movement [9]. It is well known that ACL injuries cause functional deficits due to decreased proprioception, which is one reason, that ACL-reconstruction can lead to dissatisfactory results despite technically successful surgery. Many studies report proprioceptive deficits in case of ACL-insufficiency [10]. After ACL-reconstruction, authors report heterogeneous results, some observed good restoration of proprioceptive knee function [11,12], others report persistent deficits compared to uninjured knees [13,14].

The morphological correlates for these mechanosensory characteristics of the ligament, are numerous nerve fibers, as well as Ruffini end organs and Pacinian corpuscles, which represent mechanoreceptors and account for around 2.5% of the ligaments' volume [15]. Because these mechanoreceptors can regularly be detected in the tibial ACL stump or remnants of the torn ligament, some authors plead for surgical techniques, which preserve these structures. Preservation should result in better proprioception and better clinical results [16,17], despite increasing the risk for cyclops lesions and arthrofibrosis [18]. From a pathophysiological point of view the proprioceptive features of the ACL are still not completely understood. In this context, primary cilia (PC) could be of interest. PC represent multifunctional organelles, which hair-like project from the cell and play a significant role in the signaling between the intra- and extracellular space. PC typically contain 9 microtubular doublets (9 + 0 arrangement) compared to the 9 + 2 configuration of motile cilia, which also contain several other structures, such as dynein arms [19]. This tubular structure grows out of the "basal body" (BB), which is located underneath the cell membrane and shows a cylindrical shape [20]. Ciliary associated pathways play a crucial role in the coordination of osteo- and chondrogenesis. Furthermore, the presence of PC is well known in hyaline articular cartilage, where PC appear in one per cell and show a zone-dependent orientation [21]. PC seem to be involved in pathological key mechanisms in the development of OA, such as the up-regulation of MMP-13, an enzyme, which cleaves Collagen II in hyaline cartilage [22]. Experimental studies demonstrated that the number and length of PC increase with severity of OA in the superficial zone of hyaline cartilage. Furthermore, in healthy articular cartilage, the extracellular matrix (ECM) is orientated in PC direction, which indicates that cilia are involved in orientation of collagen fibers or are orientated by the fibers [23]. Beside in the hyaline articular cartilage, PC occur in tenocytes on a regular basis, showing the same orientation as collagen fibers [24]. Despite the fact, that cilia are known for their function in mechanosensory cells in several animals [25], they have not been described in the human ACL yet. Thus, the question arises as to how the occurrence and structure of PC affect the specific tissue architecture and homeostasis of the ACL and whether any structural features of PC in the human ACL could play a role in physiological and pathophysiological processes of this tissue. In the present study, we analyzed, if PC occur in the ACL and their distribution and orientation with view to their potential function in pathophysiology.

2. Material and methods

2.1. Tissue collective

In total, seven human ACL's underwent transmission electron microscopical analysis. Three cadaveric ACL's and four freshly injured ACL's were examined. Cadaveric ACL's were obtained from the course for macroscopical anatomy from the Centre for Anatomy of the medical faculty of Cologne. In the injured group, age varied between 26 and 39 years, whereas the intact ACL, specimens came from cadavers with an age between 71 and 79 years. Body donors donated their body for medical education and medical science and signed informed consent during their lifetime. Injured ACL's were sent for diagnostic routine analyses to the Institute of Pathology of the University Regensburg. All specimens were embedded in 4% buffered formalin. This study was approved by the ethical review board of the University of Cologne (Ref.-number: 19–1642).

2.2. Transmission electron microscopy

For transmission electron microscopic analysis, tissue samples of human ACL's were transferred from their primary fixative to a buffered glutaraldehyde solution (2.5%). Following sufficient fixation, the samples were embedded automatically according to a standard protocol by use of the LYNX microscopy tissue processor (Reichert-Jung, Wetzlar, Germany). This process involved post-fixation with osmium tetroxide, dehydration and infiltration with EPON, respectively. Semi-thin sections (0,75 μm) were cut using the Reichert Ultracut S Microtome (Leica-Reichert, Wetzlar, Germany) and stained with toluidine blue and basic fuchsine for the selection of relevant areas via light microscopy. Ultrathin sections (80 nm), which were cut using the same microtome, were then contrasted with aqueous 2%-uranyl-acetate- and 2%-lead-citrate solution for 10 min each. Electron microscopy was performed using the LEO 912AB electron microscope (Zeiss, Oberkochen, Germany), equipped with a side-mounted 2kx2k CCD-Camera (TRS Tröndle, Moorenweis, Germany). In total, 276 cells of the cadaveric ACL's and 180 cells of the injured ACL's were examined. Cells were examined in their totality including the cell membrane and the nearby extracellular space regarding basal bodies and axonemes, which represent components of a PC. The cells, which showed these structures as well as the structures themselves, were counted.

2.3. Statistical analyses

A comparison of both groups was made using Dunn's multiple comparisons test. Data was collected using Microsoft Excel (Microsoft, Redmond, USA). Statistical analysis was performed using SPSS (IBM SPSS, Armonk, USA). A p-value $< .05$ was regarded as statistically significant.

3. Results

Results are given in Table 1 and Fig. 1. In total, basal bodies could be detected in three of the four specimens of the injured ACL's as well as in one of the three cadaveric ACL's. In the cadaver group, 276 cells were examined in total. While two specimens did not show any positivity, one ACL showed 7 positive cells of 160 cells counted, resulting in a positivity of 4.38% for this specimen and a positive rate of 2.54% for the group. In the group representing the injured ACL's, ciliary structures could be detected in three of the four specimens examined, with positive rates varying between 1.1% and 4.69% for the specimen showing positivity. One specimen did not show any ciliary structures, resulting in a positive rate of 2.78% in total in this group. In case of PC-presence, only one ciliary structure per cell could be detected in the cadaver-group as well

Table 1
Results of the transmission electron microscopical analysis.

	Observed cells in total	Number of basal bodies	Percentage
Cadaver 1	56	0	0%
Cadaver 2	160	7	4.38%
Cadaver 3	60	0	0
Total Cadaver	**276**	**7**	**2.54%**
Inury 1	4	0	0%
Inury 2	90	1	1.1%
Inury 3	64	3	4.69%
Inury 4	22	1	4.55%
Total Inury	**180**	**5**	**2.78%**

Cells of the ACL's were examined regarding structures indicating the presence of primary cilia (basal bodies, axonemes).

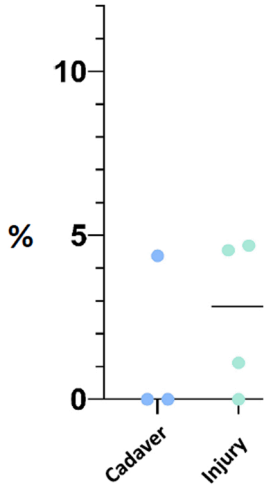

Fig. 1. Percentage of positive cells within the examined cells in cadaveric and injured ACLs. Description of Fig. 1: In the cadaver group, two specimens did not show any positivity, one ACL showed 7 positive cells of 160 counted, resulting in a positivity of 4.38% for this specimen and a positive rate of 2.54% for the group. In the injured group, ciliary structures could be detected in three of the four specimens examined, with positive rates varying between 1.1% and 4.69% for the specimen showing positivity. One specimen did not show any ciliary structures, resulting in a positive rate of 2.78% in total in this group.

as in the injury-group. No statistically significant difference regarding the frequency could be detected between both groups (cadaveric ACL's vs. injured ACL's; $p = .96$). In total, 12 basal bodies could be identified in both groups, while no axonemes were found (Fig. 2).

4. Discussion

In the present pilot-study, we identified for the first time morphological correlates of primary cilia in human ACLs by use of ultrastructural techniques. To our knowledge, the occurrence and structure of PC in the human ACL has never been studied in detail before. Based on our structural analysis, these results could provide the basis for further investigations not only with view to their physiological but also to their pathophysiological role in the human ACL.

Mechanically, the ACL avoids the anterior translation of the tibia and is involved in the limitation of tibial internal rotation [1]. Furthermore, the ACL is of crucial importance in proprioception of the knee. In this context, the ACL is part of a neurosensory network, which delivers information about joint position and joint movement. To fulfill this task, the ACL must be able to receive and process information regarding different load-conditions and different state of motions. In case of ACL-insufficiency (e.g., partial or complete rupture with damage to the nerval components), a deficit in proprioception can be observed commonly and has been described by several authors [10]. Based on our findings, future investigations should address the potential role of PC in the cellular mechanisms of these processes. PCs are present in almost all non-hematopoietic tissues of the human body, but numerous tissue-specific functions of PCs are not yet completely understood [26]. In hyaline cartilage for example, 'mechanotransduction' is one important function, namely the conversion of mechanical forces into a cellular response with view to maintain the ECM homeostasis [21,27,28].

The primary aim of our study was to clarify whether PC are present in human cruciate ligaments. In this context, in mesenchymal tissues PCs could already be identified with species- and tissue-specific differences. Wilsman et al. demonstrated rates of 100% in murine and 96% in equine chondrocytes by electron microscopy [29]. In bovine articular chondrocytes McGlashan et al. found PC incidences of 46% [23]. A few studies have addressed similar issues with a focus on other connective tissues (tendons, discus, meniscus). However, these analyses were focused on animal specimens [30]. In contrast, studies on the occurrence of PC in human connective tissues are extremely rare. In human chondrocytes, Ho et al. discovered rates of 67%, which seems to be little compared to murine and equine chondrocytes. However, the authors report, that the majority of cells within chondrosarcomas and enchondromas lack primary cilia. This leads to the conclusion, that the presence of PC suppresses the neoplastic phenotype [31]. Until now, there are no analyses regarding the presence of PC in the ACL. Nevertheless, it is of special interest to know more about the existence and special role of PC in the human ACL since the upright, bipedal walk of the human being compared to the quadrupedal gait of the mostly examined animals may

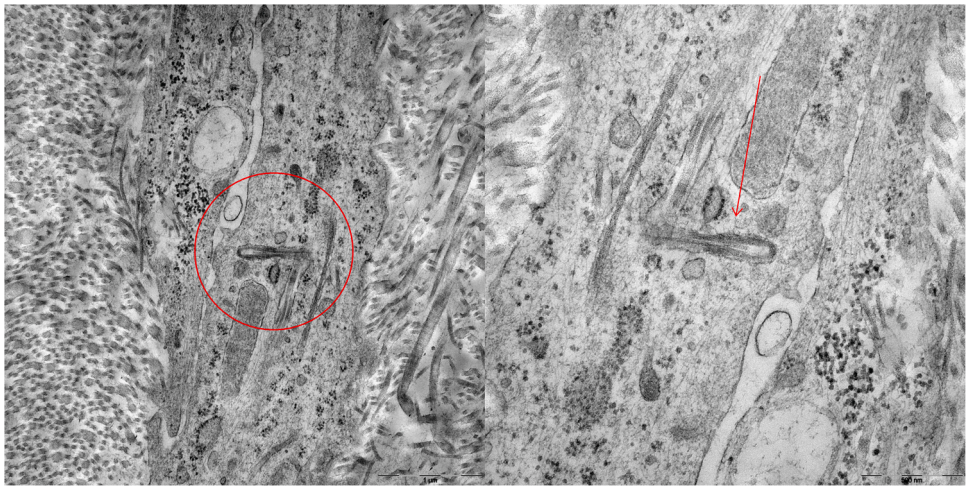

Fig. 2. Basal bodies in human ACL-cells.

be one key factor for a relevant functional difference that could significantly affect the structure and role of PC in the human ACL. Assuming a structure-function relationship, it would also be conceivable that ciliary functions could play a minor role in the ACL, such that ligamentocytes are not dependent on the presence and function of a PC. The question of whether cells of mesenchymal tissues require a PC at all or elongate it into the ECM for a functioning tissue bond could be based on differences in the structural and functional demands of the respective tissues. Hyaline cartilage tissue, for example, is a bradytrophic tissue with no nerve endings and no vascularization, whereas the ACL is permeated by vessels and nerves. Moreover, there are highly specialized mechanoreceptors (Golgi tendon receptors, Ruffini receptors, Vater-Pacini receptors, free nerve endings) in the ACL that are absent in cartilage tissue [32]. Thus, evolutionarily, the mechanotransductive property of PC may have become more established in cartilage tissue as a possible adaptation mechanism to mechanical stresses without proper blood perfusion or nervous innervation. Looking more closely at functional differences, cartilage tissue is primarily exposed to compressive loads, whereas ligament and tendon tissue are more likely to withstand tensile loads. It is conceivable that PC and its mechanosensitive functions play only a subordinate role in tissues exposed to tensile loads, since there are specific mechanosensitive receptors, which primarily take over mechanosensation and ensure tissue adaptation via reflexes and muscular control. These hypotheses should be addressed in further experimental studies.

However, differences in histologic structure and function can also be found in a direct comparison between tendon and ligament tissue. In tendon tissue, higher PC incidences and also elongated cilia and special cilia orientations were found compared to our results [24]. From a functional point of view, tendons fulfill a stabilizing function, but it also has the task of storing and releasing dynamic energy in order to convert it into highly effective movements [33]. In contrast to this dynamic function, the ligamentous tissue of the ACL primarily fulfills a more static holding and stabilizing function. This difference in physical influences could represent an important factor regarding the occurrence of PC in connective tissues and could explain the lower BB incidences and the absence of elongated cilia in our study. With that in mind, our findings support the different characteristic of tendon and ligament tissue. The mechanotransductive property of PC in cartilage tissue leads to an adaptation of the ECM of the tissue to improve the buffer function and thus better attenuation of mechanical compressive loads [29]. In tendon tissue, mechanotransductive adaptation to the more dynamic tensile loads is equally conceivable. Adaptive processes to mechanical loads are also likely in the ACL, but based on our observations, PC appear to play a minor role in this context compared to tissues subjected to more dynamic loads.

The fact that higher PC occurrences were found in the tendon and ligament tissue of other species could also be due to anatomical and physiological differences, which could be explained, for example, by the different gait patterns (upright gait vs. quadrupedal gait) and consequently different mechanical stresses.

However, it is not only the presence of PC in the corresponding tissues that is of importance. The orientation of the elongated cell organelles can also provide information about structural and functional relationships. Donnelly et al. were able to show by multiphoton microscopic studies that in tendons the PCs align along the direction of the collagen fibrils [24]. Whether this directional relationship represents an active or passive mechanism remains unanswered so far. To elucidate the physiological conditions, we examined post-mortem intact ACLs in three human cadavers. According to the question of the potential pathophysiological role of PC, we examined four surgical specimens of ACL stumps after ligament rupture and consecutive resection. Here we expected to discover special structural features of PC and to be able to relate them to possible degenerative processes, as it could be shown for example in articular cartilage affected by osteoarthritis [23]. The surgical specimens from the ACL rupture showed an age between 26 and 39 years, whereas the intact ACL specimens came from cadavers with a higher age between 71 and 79 years.

Furthermore, we focused our question regarding the physiological occurrence and incidences of PC in ligamentocytes of intact ACLs. Here, our electron microscopic study was able to show that subciliar structures (Basal Bodies = BB) can be found in cells of intact ACL tissue, which represent the basis, or key organelle, for the potential formation and elongation of PC [34,35]. However, a comparatively low occurrence of ciliary structures of only 2.54% was found in the 276 ligamentocytes examined. This corresponds, for example, to a significantly lower rate compared to human hyaline cartilage tissue [31]. Therefore, this observation might represent a tissue-specific characteristic of ACL tissue. Furthermore, the question arises whether a pathophysiological role of PC for the ACL can also be inferred via PC incidences and ciliary structures. However, in the ligament rupture group, only 5 out of a total of 180 examined cells showed BBs, corresponding to an incidence of subciliar structures of only 2.78%. Basically, we could show that in the human ACL only a small number of PC or associated BB could be detected. In this context, it is largely unclear why in the tissue network some cells form PCs and others do not. One possible explanation could be that some ligament cells tend to perform regulatory functions that are associated with the proper structure and function of a PC. An alternative hypothesis could be, that PC bearing cells are in biomolecular-sensory communication with the other cells of the tissue in the sense of a functional unit. Finally, it is possible that the cilium-bearing cells may be different cell phenotypes of e.g., ligamentocytes, or even progenitor cells or stem cells resident in the tissue matrix, which are able to sense the microenvironment by means of the PC and respond to changes with cell division, proliferation and differentiation to consequently ensure the maintenance of tissue homeostasis. It is well known, that especially in bone marrow derived mesenchymal stem cells, PC is commonly present [36]. These hypotheses need a further scientific proof, which could change our understanding of ACL structure and function relationship.

Interestingly, our results revealed, that not a single one of the BB-containing cells carries an elongated PC. This raises the question whether this represents a species- or tissue-specific property or a physiological adaptation mechanism to certain external circumstances or even pathological changes. Physiologically, PCs have the ability to adapt to external circumstances by means of changes in length [37]. In cartilage tissue, for example, it has been shown that there is a pathophysiological interaction of osteoarthritic processes with a change in length of the PC [38]. However, the fact that no elongated PC could be found in the BB-bearing ligamentocytes of the intact ACLs examined was surprising. This would imply that physiologically in the mature ACL, elongated PCs are not necessary for proper tissue function and homeostasis. Furthermore, genetic factors during ACL tissue growth and differentiation might prevent PC formation. In this context, it is a matter to be analyzed more in detail whether PCs play a role during embryological development in this tissue type, as it has been shown in cartilage and bone tissue [39,40]. Therefore, the influence of external and genetic factors on embryonic and postpartum maturation processes should be further investigated.

Our findings revealed no significant difference in the occurrence or structure of PC between the intact ACL tissue and the ruptured ligaments. The lack of difference in the cells examined between the two groups may suggest that our observations are more likely a tissue-specific feature. Considering the pathomechanisms of ACL rupture, which can also occur in healthy ACLs due to a short-term massive overload without prior degenerative changes of the ligament, it would be possible that the lack of difference in the studied groups is due to lack of preexisting degenerative changes of the ligaments. Thus, a closer look at ciliary structures and specificities in degenerative pre-modified ACLs should also be considered in the future. However, on the basis of our study results, pathophysiological correlations regarding PC and ligament ruptures seem to play a rather minor role.

Using hyaline articular cartilage as an example, it has been shown

that in the superficial zone, i.e., the area of greatest force application, the PCs continuously point away from the articular cartilage surface in the direction of applied forces and elongate into the ECM below the cell body of the chondrocytes [41]. Based on this observation, it could be postulated that PCs align passively, i.e., according to the direction of the forces acting on the tissue. This might be reasonable from a pathophysiological point of view, since alignment of the cilium in an orthogonal direction to the applied force would expose the PC to severe shear stress, causing possible overstimulation of the ciliary apparatus or even structural damage. On the other hand, the direction of the cilium protruding from the cell body could also be actively controlled by the cell, for example, to ensure a certain tissue organization by means of cell rotations and ECM modulations. Here Andrea et al. could show that in osteochondroma-altered cartilage compared to healthy hyaline tissue the directional relationship of the cilia and the specific tissue architecture are abolished [42]. However, some limitations have to be mentioned. Tissue removal of intact ACLs from young and healthy people of similar age of the rupture group could of course not be justified from an ethical and moral point of view. Therefore, unfortunately, age-specific correlations in the groups cannot be excluded with respect to our study results. Furthermore, the occurrence and structure of PC might also change with age. However, this would need to be investigated in more detail in other contexts.

5. Conclusions

In summary, for the first time we identified BB as a correlate for PC in the human ACL. Furthermore, we found no elongated cilia and only minor occurrences of subciliary structures (BBs) in human ACL tissue by our ultrastructural analysis of PC. Based on our results, it can be considered that, compared with studies of other species and other connective tissues, this observation may be a species and tissue-specific feature. The absence of elongated cilia also suggests a subordinate role of these cell organelles in human ACLs. The comparison with studies on the structure and function of PCs in other connective tissues opens up new, innovative perspectives regarding the physiological and pathological role of these multisensory cellular organelles in the ACL. Whether our investigated morphological features are an expression of physiological peculiarities or pathological alterations cannot be definitively clarified by our investigation and requires further studies of PC in the context of the corresponding pathologies. In particular, the structure and function of PC should also be investigated in more detail in ACL reconstructions from tendon tissue to investigate alternative adaptive mechanisms with the mechanotransductive property of PC after the reduction of proprioceptive abilities.

CRediT authorship contribution statement

David Grevenstein: Conceptualization, Investigation, Formal analyses, Validation, Visualization, Writing – review & editing. **Johannes Oppermann**: Conceptualization, Writing – review & editing. **Lina Winter**: Validation, Visualization, Writing – review & editing. **Friedrich Barsch**: Investigation, Formal analyses. **Tanja Niedermair**: Investigation, Formal analyses, Supervision, Writing – review & editing. **Andreas Mamilos**: Investigation, Formal analyses. **Peer Eysel**: Conceptualization, Supervision, Writing – review & editing. **Christoph Brochhausen**: Conceptualization, Investigation, Formal analyses, Supervision, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] H. Matsumoto, Y. Suda, T. Otani, Y. Niki, B.B. Seedhom, K. Fujikawa, Roles of the anterior cruciate ligament and the medial collateral ligament in preventing valgus instability, J. Orthop. Sci. 6 (2001) 28–32, https://doi.org/10.1007/s007760170021.

[2] A.A. Amis, G.P. Dawkins, Functional anatomy of the anterior cruciate ligament. Fibre bundle actions related to ligament replacements and injuries, J. Bone Jt. Surg. Br. 73 (1991) 260–267, https://doi.org/10.1302/0301-620X.73B2.2005151.

[3] W. Petersen, T. Zantop, Anatomy of the anterior cruciate ligament with regard to its two bundles, Clin. Orthop. Relat. Res. 454 (2007) 35–47, https://doi.org/10.1097/BLO.0b013e31802b4a59.

[4] S.P. Arnoczky, Anatomy of the anterior cruciate ligament, Clin. Orthop. Relat. Res. (1983) 19–25.

[5] L.Y. Griffin, M.J. Albohm, E.A. Arendt, R. Bahr, B.D. Beynnon, M. Demaio, R. W. Dick, L. Engebretsen, W.E. Garrett, J.A. Hannafin, T.E. Hewett, L.J. Huston, M. L. Ireland, R.J. Johnson, S. Lephart, B.R. Mandelbaum, B.J. Mann, P.H. Marks, S. W. Marshall, G. Myklebust, F.R. Noyes, C. Powers, C. Shields, S.J. Shultz, H. Silvers, J. Slauterbeck, D.C. Taylor, C.C. Teitz, E.M. Wojtys, B. Yu, Understanding and preventing noncontact anterior cruciate ligament injuries: a review of the Hunt Valley II meeting, January 2005, Am. J. Sports Med. 34 (2006) 1512–1532, https://doi.org/10.1177/0363546506286866.

[6] D.P. Piasecki, K.P. Spindler, T.A. Warren, J.T. Andrish, R.D. Parker, Intraarticular injuries associated with anterior cruciate ligament tear: findings at ligament reconstruction in high school and recreational athletes. An analysis of sex-based differences, Am. J. Sports Med. 31 (2003) 601–605, https://doi.org/10.1177/03635465030310042101.

[7] B.T. Samuelsen, K.E. Webster, N.R. Johnson, T.E. Hewett, A.J. Krych, Hamstring autograft versus patellar tendon autograft for ACL reconstruction: is there a difference in graft failure rate? A meta-analysis of 47,613 patients, Clin. Orthop. Relat. Res. 475 (2017) 2459–2468, https://doi.org/10.1007/s11999-017-5278-9.

[8] B. Luc, P.A. Gribble, B.G. Pietrosimone, Osteoarthritis prevalence following anterior cruciate ligament reconstruction: a systematic review and numbers-needed-to-treat analysis, J. Athl. Train. 49 (2014) 806–819, https://doi.org/10.4085/1062-6050-49.3.35.

[9] M.S. Dhillon, K. Bali, S. Prabhakar, Proprioception in anterior cruciate ligament deficient knees and its relevance in anterior cruciate ligament reconstruction, Indian J. Orthop. 45 (2011) 294–300, https://doi.org/10.4103/0019-5413.80320.

[10] J.P. Corrigan, W.F. Cashman, M.P. Brady, Proprioception in the cruciate deficient knee, J. Bone Jt. Surg. Br. 74 (1992) 247–250, https://doi.org/10.1302/0301-620X.74B2.1544962.

[11] Q.I. Muaidi, L.L. Nicholson, K.M. Refshauge, R.D. Adams, J.P. Roe, Effect of anterior cruciate ligament injury and reconstruction on proprioceptive acuity of knee rotation in the transverse plane, Am. J. Sports Med. 37 (2009) 1618–1626, https://doi.org/10.1177/0363546509332429.

[12] A.G. Angoules, A.F. Mavrogenis, R. Dimitriou, K. Karzis, E. Drakoulakis, J. Michos, P.J. Papagelopoulos, Knee proprioception following ACL reconstruction; a prospective trial comparing hamstrings with bone-patellar tendon-bone autograft, Knee 18 (2011) 76–82, https://doi.org/10.1016/j.knee.2010.01.009.

[13] J.O. Anders, R.A. Venbrocks, M. Weinberg, Proprioceptive skills and functional outcome after anterior cruciate ligament reconstruction with a bone-tendon-bone graft, Int. Orthop. 32 (2008) 627–633, https://doi.org/10.1007/s00264-007-0381-2.

[14] T.R. Bonfim, C.A. Jansen Paccola, J.A. Barela, Proprioceptive and behavior impairments in individuals with anterior cruciate ligament reconstructed knees11No commercial party having a direct financial interest in the results of the research supporting this article has or will confer a benefit upon the authors(s) or upon any organization with which the author(s) is/are associated, Arch. Phys. Med. Rehabil. 84 (2003) 1217–1223, https://doi.org/10.1016/S0003-9993(03)00147-3.

[15] M.L. Zimny, M. Schutte, E. Dabezies, Mechanoreceptors in the human anterior cruciate ligament, Anat. Rec. 214 (1986) 204–209, https://doi.org/10.1002/ar.1092140216.

[16] Z. Li, L. Zhang, Current concepts in arthroscopic reconstruction of anterior cruciate ligament with remnant preservation technique, Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi 24 (2010) 304–308.

[17] B.-I. Lee, K.-D. Min, H.-S. Choi, J.-B. Kim, S.-T. Kim, Arthroscopic anterior cruciate ligament reconstruction with the tibial-remnant preserving technique using a hamstring graft, 340, Arthroscopy 22 (2006) e1–e7, https://doi.org/10.1016/j.arthro.2005.11.010.

[18] H.O. Mayr, T.G. Weig, W. Plitz, Arthrofibrosis following ACL reconstruction–reasons and outcome, Arch. Orthop. Trauma Surg. 124 (2004) 518–522, https://doi.org/10.1007/s00402-004-0718-x.

[19] P. Satir, L.B. Pedersen, S.T. Christensen, The primary cilium at a glance, J. Cell Sci. 123 (2010) 499–503, https://doi.org/10.1242/jcs.050377.

[20] F. Barsch, T. Niedermair, A. Mamilos, V.H. Schmitt, D. Grevenstein, M. Babel, T. Burgoyne, A. Shoemark, C. Brochhausen, Physiological and pathophysiological aspects of primary cilia-a literature review with view on functional and structural relationships in cartilage, Int. J. Mol. Sci. 21 (2020), https://doi.org/10.3390/ijms21144959.

[21] C.G. Jensen, C.A. Poole, S.R. McGlashan, M. Marko, Z.I. Issa, K.V. Vujcich, S. S. Bowser, Ultrastructural, tomographic and confocal imaging of the chondrocyte primary cilium in situ, Cell Biol. Int. 28 (2004) 101–110, https://doi.org/10.1016/j.cellbi.2003.11.007.

[22] I.D. Sheffield, M.A. McGee, S.J. Glenn, Y. Da Baek, J.M. Coleman, B.K. Dorius, C. Williams, B.J. Rose, A.E. Sanchez, M.A. Goodman, J.M. Daines, D.L. Eggett, V.

C. Sheffield, A. Suli, D.L. Kooyman, Osteoarthritis-like changes in bardet-biedl syndrome mutant ciliopathy mice (Bbs1M390R/M390R): evidence for a role of primary cilia in cartilage homeostasis and regulation of inflammation, Front. Physiol. 9 (2018) 708, https://doi.org/10.3389/fphys.2018.00708.
[23] S.R. McGlashan, E.C. Cluett, C.G. Jensen, C.A. Poole, Primary cilia in osteoarthritic chondrocytes: from chondrons to clusters, Dev. Dyn. 237 (2008) 2013–2020, https://doi.org/10.1002/dvdy.21501.
[24] E. Donnelly, M.-G. Ascenzi, C. Farnum, Primary cilia are highly oriented with respect to collagen direction and long axis of extensor tendon, J. Orthop. Res. 28 (2010) 77–82, https://doi.org/10.1002/jor.20946.
[25] A. Jarman, P. zur Lage, G. Mali, P. Mill, The regulation of mechanosensory motile cilium formation, Cilia 4 (2015) O7, https://doi.org/10.1186/2046-2530-4-S1-O7.
[26] J.M. Brown, G.B. Witman, Cilia and diseases, Bioscience 64 (2014) 1126–1137, https://doi.org/10.1093/biosci/biu174.
[27] K.K. Papachroni, D.N. Karatzas, K.A. Papavassiliou, E.K. Basdra, A. G. Papavassiliou, Mechanotransduction in osteoblast regulation and bone disease, Trends Mol. Med. 15 (2009) 208–216, https://doi.org/10.1016/j.molmed.2009.03.001.
[28] R. Ruhlen, K. Marberry, The chondrocyte primary cilium, Osteoarthr. Cartil. 22 (2014) 1071–1076, https://doi.org/10.1016/j.joca.2014.05.011.
[29] N.J. Wilsman, C.E. Farnum, D.K. Reed-Aksamit, Incidence and morphology of equine and murine chondrocytic cilia, Anat. Rec. 197 (1980) 355–361, https://doi.org/10.1002/ar.1091970309.
[30] E. Donnelly, R. Williams, C. Farnum, The primary cilium of connective tissue cells: imaging by multiphoton microscopy, Anat. Rec. 291 (2008) 1062–1073, https://doi.org/10.1002/ar.20665.
[31] L. Ho, S.A. Ali, M. Al-Jazrawe, R. Kandel, J.S. Wunder, B.A. Alman, Primary cilia attenuate hedgehog signalling in neoplastic chondrocytes, Oncogene 32 (2013) 5388–5396, https://doi.org/10.1038/onc.2012.588.
[32] V.B. Duthon, C. Barea, S. Abrassart, J.H. Fasel, D. Fritschy, J. Ménétrey, Anatomy of the anterior cruciate ligament, Knee Surg. Sports Traumatol. Arthrosc. 14 (2006) 204–213, https://doi.org/10.1007/s00167-005-0679-9.
[33] A.D. Foster, B. Block, F. Capobianco, J.T. Peabody, N.A. Puleo, A. Vegas, J. W. Young, Shorter heels are linked with greater elastic energy storage in the Achilles tendon, Sci. Rep. 11 (2021) 9360, https://doi.org/10.1038/s41598-021-88774-8.
[34] G. Garcia, D.R. Raleigh, J.F. Reiter, How the ciliary membrane is organized inside-out to communicate outside-in, Curr. Biol. 28 (2018) R421–R434, https://doi.org/10.1016/j.cub.2018.03.010.
[35] W.F. Marshall, What is the function of centrioles? J. Cell. Biochem. 100 (2007) 916–922, https://doi.org/10.1002/jcb.21117.
[36] P. Tummala, E.J. Arnsdorf, C.R. Jacobs, The role of primary cilia in mesenchymal stem cell differentiation: a pivotal switch in guiding lineage commitment, Cell. Mol. Bioeng. 3 (2010) 207–212, https://doi.org/10.1007/s12195-010-0127-x.
[37] D.R. Rich, A.L. Clark, Chondrocyte primary cilia shorten in response to osmotic challenge and are sites for endocytosis, Osteoarthr. Cartil. 20 (2012) 923–930, https://doi.org/10.1016/j.joca.2012.04.017.
[38] S.R. McGlashan, M.M. Knight, T.T. Chowdhury, P. Joshi, C.G. Jensen, S. Kennedy, C.A. Poole, Mechanical loading modulates chondrocyte primary cilia incidence and length, Cell Biol. Int. 34 (2010) 441–446, https://doi.org/10.1042/CBI20090094.
[39] B. Song, C.J. Haycraft, H. Seo, B.K. Yoder, R. Serra, Development of the post-natal growth plate requires intraflagellar transport proteins, Dev. Biol. 305 (2007) 202–216, https://doi.org/10.1016/j.ydbio.2007.02.003.
[40] C.J. Haycraft, Q. Zhang, B. Song, W.S. Jackson, P.J. Detloff, R. Serra, B.K. Yoder, Intraflagellar transport is essential for endochondral bone formation, Development 134 (2007) 307–316, https://doi.org/10.1242/dev.02732.
[41] C.E. Farnum, N.J. Wilsman, Orientation of primary cilia of articular chondrocytes in three-dimensional space, Anat. Rec. 294 (2011) 533–549, https://doi.org/10.1002/ar.21330.
[42] C.E. de Andrea, M. Wiweger, F. Prins, J.V.M.G. Bovée, S. Romeo, P.C. W. Hogendoorn, Primary cilia organization reflects polarity in the growth plate and implies loss of polarity and mosaicism in osteochondroma, Lab. Investig. 90 (2010) 1091–1101, https://doi.org/10.1038/labinvest.2010.81.