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Anterior cruciate ligament: an anatomical exploration in humans and in a selection of animal species

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Abstract

Purpose Many anatomical anterior cruciate ligament (ACL) studies have indicated that the human ACL is composed of two functional bundles: the antero-medial (AM) and postero-lateral (PL). The purpose of this study is to compare the ACL anatomy among human and assorted animal species.

Methods Twenty fresh-frozen knees specimen were used: five humans, ten porcine, one goat, one Kodiak bear, one African lion, one Diana monkey and one Gazelle antelope. All the specimens were dissected to expose the ACL and to visualize the number of bundles and attachment patterns on the tibia and femur. Following the fibre orientation of the individual bundles, a wire loop was used to bluntly separate the bundles starting from the tibial insertion site to the femoral insertion site. In the human and porcine ACL, each bundle was separated into approximately 2 mm diameter segments and then tracked in order to establish the individual bundle's specific pattern of insertion on the femur and tibia.

Results It appeared that all human and animal knee specimens had three bundles that made up their ACL. In addition, it was noted that among the various specimens species, all viewed with an anterior view, and at 90° knee flexion, the ACL bony insertion sites had similar attachment patterns.

Conclusion In all the specimens, including human, the ACL had three distinct bundles: AM, intermediate (IM) and PL. The bundles were composed of multiple fascicles arranged in a definite order and similar among the different species.

Keywords Three bundles · Intermediate bundle · Insertion site pattern · Human ACL · Porcine ACL

Introduction

The anterior cruciate ligament (ACL) is one of most commonly injured ligaments of the knee [18], and understanding its complex anatomy is a requisite for any orthopaedic surgeon. Presently, a more anatomical approach to reconstructing the ACL has evoked the interest of and discussion by many surgeons, and many anatomical ACL studies have been done to better understand the ACL anatomy. Yet, with all the anatomical studies, there is still controversy in regards to the number of bundles and the insertion site patterns of the ACL.

In the literature, the ACL is reported to have one bundle [22], two bundles the AM (antero-medial) and PL (postero-lateral) [1, 2, 4, 5, 7, 11, 16, 24, 27, 33–36], three bundles the AM, IM (intermediate) and PL [3, 10, 14, 21, 23], and even up to six to ten bundles [20]. It is widely reported that the human ACL is composed of two bundles based on behaviour (the PL bundle being lax and the AM bundle being tight when the knee is flexed), as a basis for the understanding the function of the ACL [1, 2, 4, 5, 7, 11, 16, 24, 25, 27, 33–36], but these studies do not consider anatomy. The studies are not conclusive as to the number

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of bundles in that the function of a bundle was perhaps too subtle to be isolated during flexion–extension, or the function is for some other motion than flexion–extension. In other studies that looked at anatomy, three bundles of the ACL were noted; Norwood and Cross [21] were the first to describe the human ACL attachment sites for the three bundles, AM, PL and IM. Amis et al. [3] as well as Iwahashi et al. [14], Fujie et al. [10] and Otsubo et al. [23] divided the ACL in three (AM, IM and PL) bundles; however, while there was agreement on the number of bundles, there were differences in the locations of the bundle's femoral and tibial insertion sites. Therefore, the anatomy of the human adult ACL still lacks consensus [2–5, 9, 11, 16, 22, 24, 25, 33–36].

The anatomy of the ACL in animals is of interest, because animal models are sometimes used in studies of the knee [26, 31, 32] and because they may give insight into the form and function of the human ACL. Anatomical studies of porcine knees have found the porcine ACL to have three distinct bundles [15] where the anterior root of the lateral meniscus physically separates the AM from IM bundle. Comparing the ACL in humans and different animal species provides the surgeon information on different shapes and sizes of ACLs and their insertion sites. These anatomical insights would better prepare the surgeon for different ACL morphologies encountered during surgery. For these reasons, the purpose of this study is to compare the ACL anatomy among human and assorted animal specimens.

Materials and methods

Twenty fresh-frozen knee specimens were used for this anatomical study: five fresh-frozen human cadavers, all male (mean age = 57, range = 47–61); ten mature porcine; one mature goat; one adult Kodiak bear; one adult African lion; one adult Diana monkey; and one adult Gazelle antelope. All knee specimens were examined for ACL or other ligament injury. For the human cadaver knees, prior approval was obtained from our institutional review board. The bear, lion, monkey and antelope hind limbs were donated by a local zoo, after the animals had passed by natural causes. The pig and goat hind limbs were obtained from local meat markets. All the specimens in this study were dissected by the same person (GT) and assisted by the co-authors. The knee specimens were kept frozen at −20 °C and defrosted at room temperature for 24 h prior to dissection.

Dissecting technique

First the synovial and soft tissue was carefully removed, exposing the ACL bundle fibres. The septum and individual bundle fibre's orientations were identified, and any gaps in the ACL fibres were noted when the knee was moved in a passive flexion–extension path. A clear difference in fibre orientation was observed and allowed for division of the bundles. In some species, porcine, goat and antelope, the anterior root of the lateral meniscus physically separates the bundles. A Penfield elevator was gently inserted into the physically separated space between the different fibre orientated AM and IM bundles, and plastic-coated wires were passed and looped around each bundle. Following the fibre orientation of the individual bundles, a wire loop was used to bluntly separate the bundle starting from the tibial insertion site to the femoral insertion site. The Penfield elevator was then inserted between the IM and PL bundles, and a plastic-coated wire was passed and looped around the PL (Fig. 1).

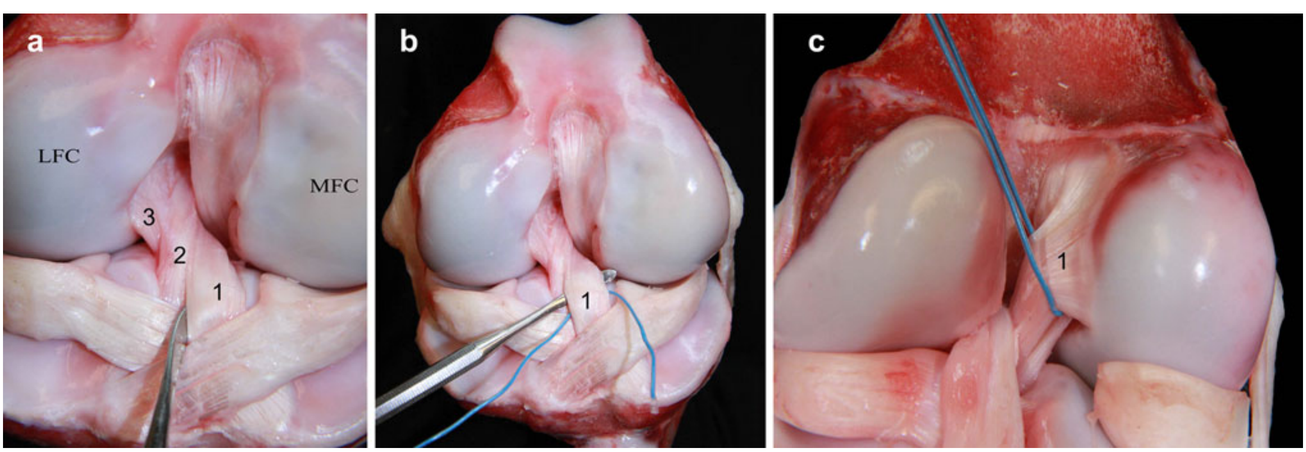


Fig. 1 Porcine knee: the standardized dissecting technique. **a** Anterior view at 90° knee flexion: a Penfield elevator was inserted into the physically separated space between the AM (1) and IM (2) bundles; PL (3). **b** A plastic-coated wire was passed and looped around the bundle. **c** Posterior view: the wire loop was used to bluntly separate the bundle starting from the tibial insertion site to the femoral insertion site

Then, the medial condyle of the femur was cut away to expose the femoral ACL insertion site to see the insertion site pattern of each bundle (Fig. 2). For all samples, each bundle was cut sequentially at the base of femoral insertion site, starting proximal to distal and then tracked carefully in order to establish the individual bundle's specific pattern of insertion on the femur and tibia. In addition, for the human and porcine specimens, after each bundle was identified, each bundle, starting with AM, then IM and finally the PL, was further separated into approximately 2 mm diameter segments. This was the smallest possible size without damage to the segment. Unfortunately, this bundle segmentation technique could only be done on the human and porcine specimens, as the other specimens were being preserved for further study. The numerous porcine specimens were used to develop the bundle segmented technique because the porcine ACL has clear distinct bundles, and the macroscopic synovial septum defines a gap, and, additionally, the anterior root of the lateral meniscus physically separates the bundles.

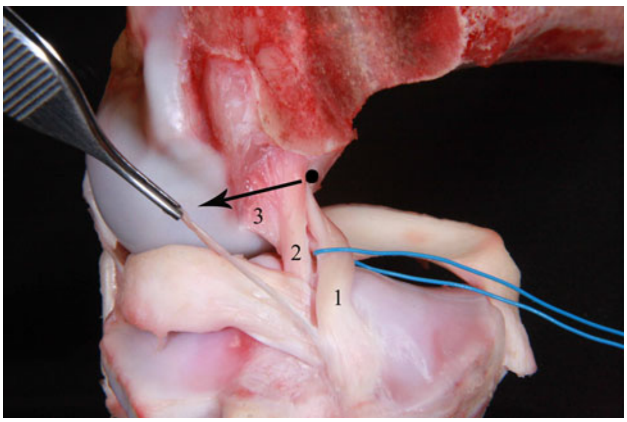


Fig. 2 Porcine knee with medial condyle removed (AM-1, IM-2 and PL-3). Starting at the *black dot*, the AM bundle at femoral insertion site is tracked (proximal to distal) in order to establish the corresponding tibial insertion site

After each of the bundles were cut from the femoral insertion site, the footprints were painted with different colours (Blue-AM, Green-IM and Red-PL) which corresponded to the coloured wire loops used during dissection (Fig. 3). The bundles were then cut from the tibial insertions, and the insertion sites were painted the appropriate colours. This dissecting technique was used to identify the human and other animal specimens' ACL bundles for comparison.

Results

In all the specimens, the ACL is composed of multiple fascicles arranged in a definite order and similar among

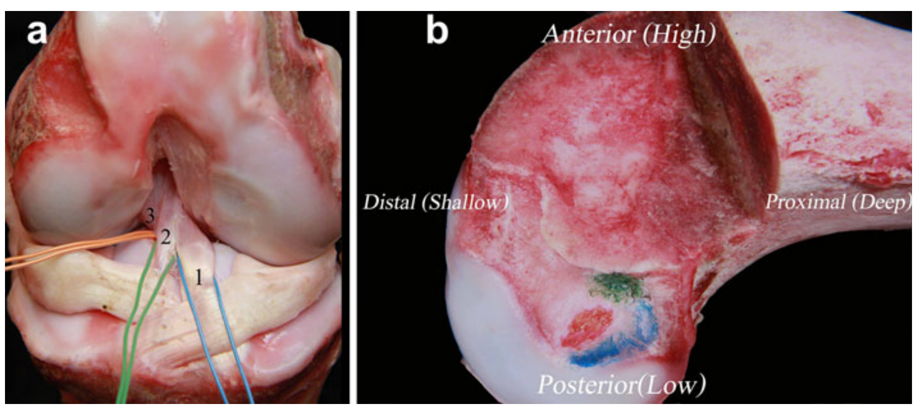
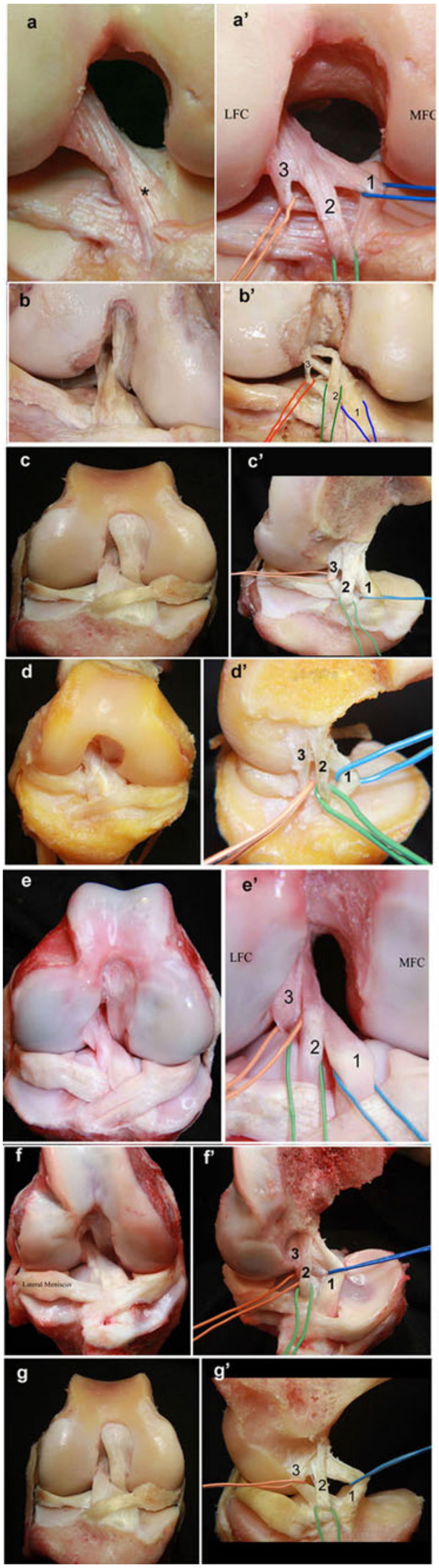


Fig. 3 Porcine Knee: **a** ACL bundles separated (AM-1, IM-2 and PL-3). **b** Femoral insertion site (*Blue*-AM, *Green*-IM and *Red*-PL). (The surgical navigation terminology described by Amis and Jacob [3]; high, low, deep and shallow)

Table 1 Summary of anatomical features of all specimens

Species	Figure 4	No. of specimens	Anterior root of the lateral meniscus separates the bundles	Gap between AM & IM	Three Different fibre orientations	No. of bundles
Human	a–a′	5	No	Yes	Yes	3
Bear	b–b′	1	No	No	Yes	3
Lion	c–c′	1	No	No	Yes	3
Monkey	d–d′	1	No	No	Yes	3
Porcine	e–e′	10	Yes	Yes	Yes	3
Goat	f–f′	1	Yes	Yes	Yes	3
Gazelle	g–g′	1	Yes	Yes	Yes	3


a
a'
LFC
MFC
3
2
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b
b'
c
c'
d
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e
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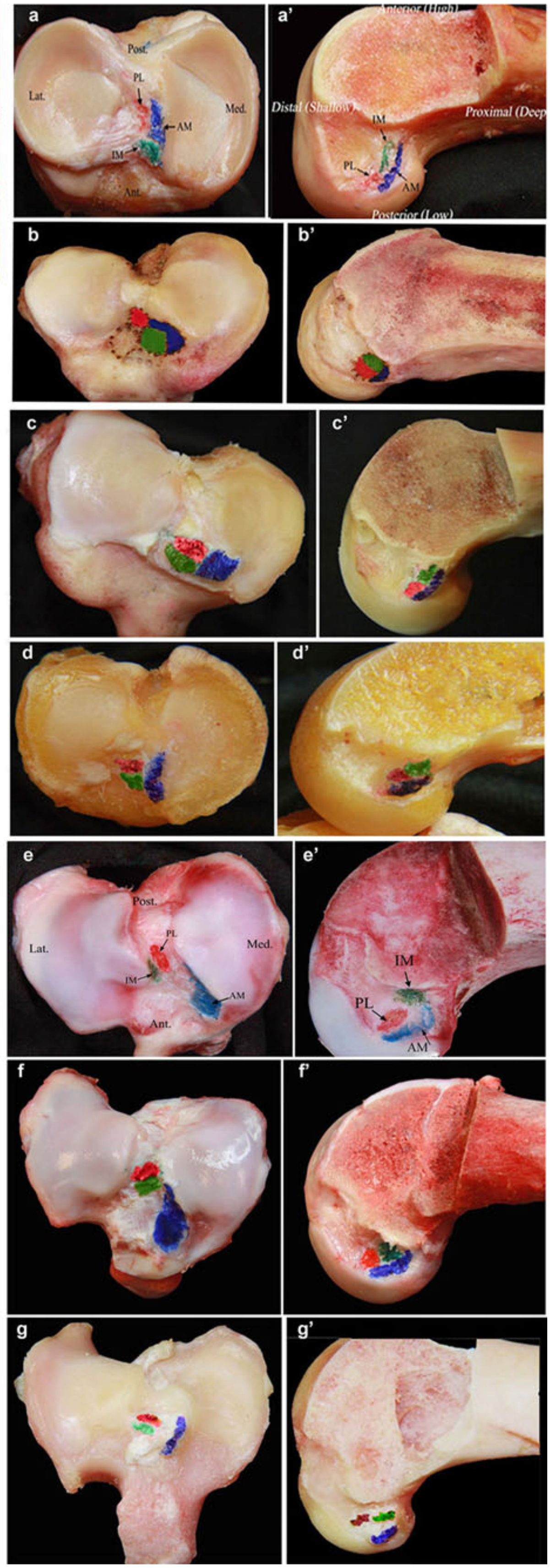
a
a'
Post.
Lat.
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Anterior (High)
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b
b'
c
c'
d
d'
e
e'
f
f'
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◀**Fig. 4** Assorted dissected specimens: (**a–a′**)-Human, (**b–b′**)-Bear, (**c–c′**)-Lion, (**d–d′**)-Monkey, (**e–e′**)-Porcine, (**f–f′**)-Goat, (**g–g′**)-Antelope. *Left column*: Anterior views at 90° of knee flexion with ACL (**a–g**) and three bundles view (**a′–g′**). *Right column*: Tibial insertion sites (**a–g**) and femoral insertion sites (**a′–g′**). *Blue*-(AM-1), *Green*-(IM-2), *Red*-(PL-3)

different species. The fibre orientation and general pattern of the ACL insertion sites are somewhat similar among the different species despite the difference in bony morphology. All the specimens, including human, appeared to have three distinct bundles for the ACL (Table 1; Fig. 4).

It was noted in the goat and antelope knee specimens, the anterior root of the lateral meniscus physically separated the AM and IM bundles on the tibial insertion, similar to the porcine knee (Fig. 5). The AM tibial insertion is separated from the IM and PL insertions. In contrast, this was not evident in the bear, lion, monkey or human knee specimens, where the anterior root of the lateral meniscus is located just lateral to the ACL attachment, and the tibial insertion of each bundle is closer together. Only the porcine knee has a macroscopic synovial septum that defined a physically separation segment between the bundles.

In all the specimens, it is noted that the AM bundle, on the femur, is located just posterior to the IM and PL bundles and expands to the rim of posterior condyle cartilage. In general, the insertion site is thin and elongated. The IM bundle stays in-line with PL bundle, right-posterior to the intercondylar ridge and anterior to AM bundle. The PL and IM femoral insertions appeared more oval, especially in the bear, lion, pig and goat.

At the tibial insertion, the AM bundle is located along the edge of the medial tibial plateau articular cartilage. The IM and PL bundles were located lateral to the AM; and the PL was generally located posterior to the IM bundle. It was noted that in the human, bear, lion and monkey, the AM, IM and PL are adjacent to each other in a triangular fashion, and in the pig, goat and antelope, the AM is slightly separated medially from the IM and PL.

In all specimens, however, from an anterior viewpoint at 90° of knee flexion, it was easy to distinguish the three different fibre orientations of the bundles (Figs. 4, 5).

Porcine knee

The AM bundle is medial to the gap between the IM and AM bundles and attaches on the AM tibial ACL insertion site, anterior to the anterior root of the lateral meniscus along the edge of the articular cartilage of the medial tibial plateau. The fibres extend to the medial intercondylar tubercle in the antero-posterior direction. At 90° of knee flexion, it twists and runs to the posterior portion of the femoral attachment from anterior and proximal (high and deep in the notch according to the surgical navigation terminology described by Amis and Jacob [4]) to posterior and distal (low and shallow) and is covered by the IM and PL bundles. The AM bundle is hidden by the vertical fibres of the IM bundle on the path up to the femur as it approaches the posterior cruciate ligament (PCL) (Figs. 5, 6a).

The IM bundle has vertical fibres lateral to the AM-IM gap (Fig. 5), and it attaches on the antero-lateral tibial ACL insertion site, posterior to the anterior root of the lateral meniscus in the antero-posterior oblique direction. It has a vertical oriented path until its attachment in the anterior and proximal (high and deep) portion of the femoral ACL insertion site, just posterior to the intercondylar ridge and anterior to upper portion of the AM bundle (Fig. 6b).

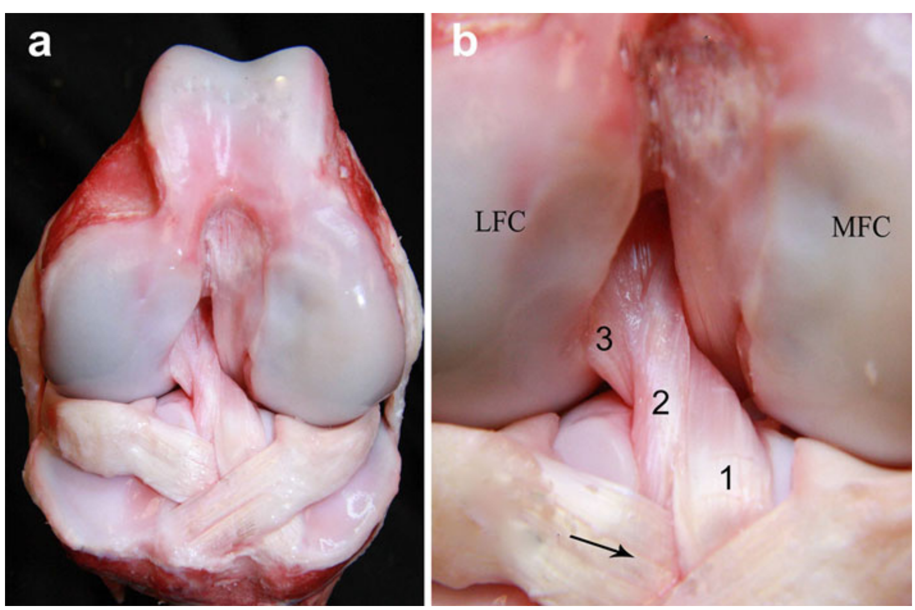

Fig. 5 Porcine knee: **a** Anterior view at 90° knee flexion **b** View of ACL between the lateral femoral condyle (LFC) and the medial femoral condyle (MFC). *Numbers* indicate the AM (1), IM (2) and PL (3) bundles and the *black arrow* points to the anterior root of the lateral meniscus that passes through a gap between the AM and IM bundle

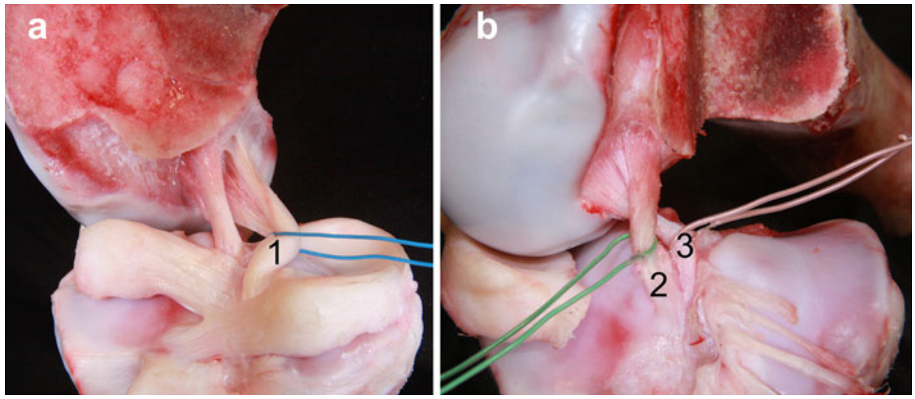

Fig. 6 Porcine knee: **a** Medial femoral condyle removed and **b** the ACL bundles separated (AM-1, IM-2 and PL-3)

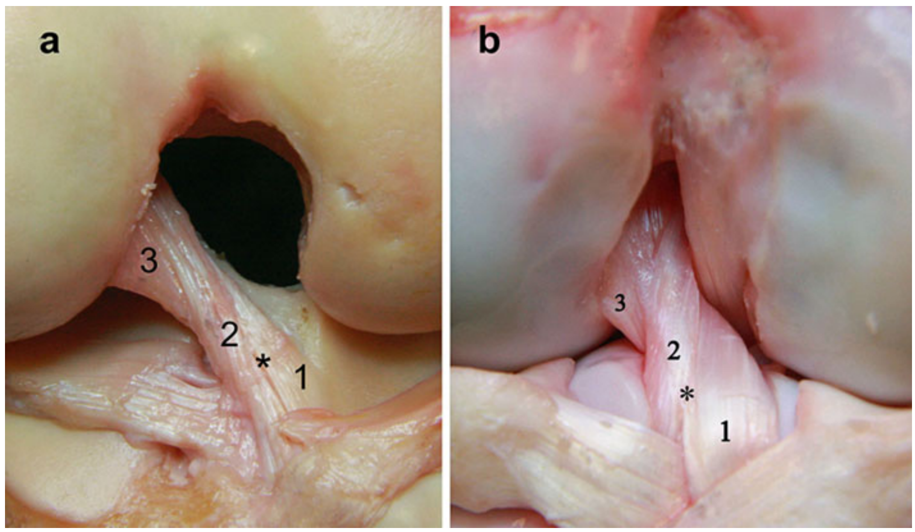

Fig. 7 Comparative Anterior view at 90° flexion (AM-1, IM-2 and PL-3): **a** human ACL **b** porcine ACL. The *asterisk* indicates the narrow fissure between the AM and IM bundle

The PL bundle's tibial attachment is posterior to the IM bundle in an antero-posterior oblique direction, is covered by the IM bundle and has a horizontal oriented path (crossing fibres) until its attachment in the posterior and distal (low and shallow) portion on the femoral ACL insertion site, in-line with the IM attachment (Fig. 6b).

Human and porcine ACL

Unlike the porcine knees, the human ACL bundles are not separated by the anterior root of lateral meniscus nor synovial septum tissue located between AM and IM. After removal of the synovial tissue covering the ACL, all of the five human specimens were found to have a narrow fissure between AM and IM bundles (Fig. 7). One of the human knee specimens was found to have fat protrusion between the AM and IM and the IM and PL bundles (Fig. 8).

When the human ACL was compared to the porcine ACL at 90° of flexion, three different fibre orientations could be seen on the human ACL; similar to what is observed in the porcine ACL (Fig. 9).

After dissecting all five human ACLs, it was noted each bundle was not round but had a ribbon-like shape, and attached to the femur in a flat, fan pattern (Figs. 9c, 10a). The IM bundle stays in-line with PL bundle, right-posterior to the intercondylar ridge and anterior to the AM bundle. On the femur, the AM bundle, just posterior to IM and PL bundles, expands to the rim of posterior condyle cartilage. The final form of the femoral insertion was in the shape of a segment of a circle. At the tibial insertion site, the insertion of each bundle fans out and forms a so-called foot region, which was different from the femoral insertion, making the tibial insertion site shape more rounded in appearance (Fig. 10c).

The AM bundle is located along the edge of the medial tibial plateau articular cartilage. The IM and PL bundles were located lateral to the AM, and the PL was generally located posterior to the IM bundle. Among the human specimens, the arrangement of each bundle at the tibial insertion site varied more widely than that of the femur, especially the PL bundle. This was also noted in the porcine specimens. The shape and size of tibial insertion site

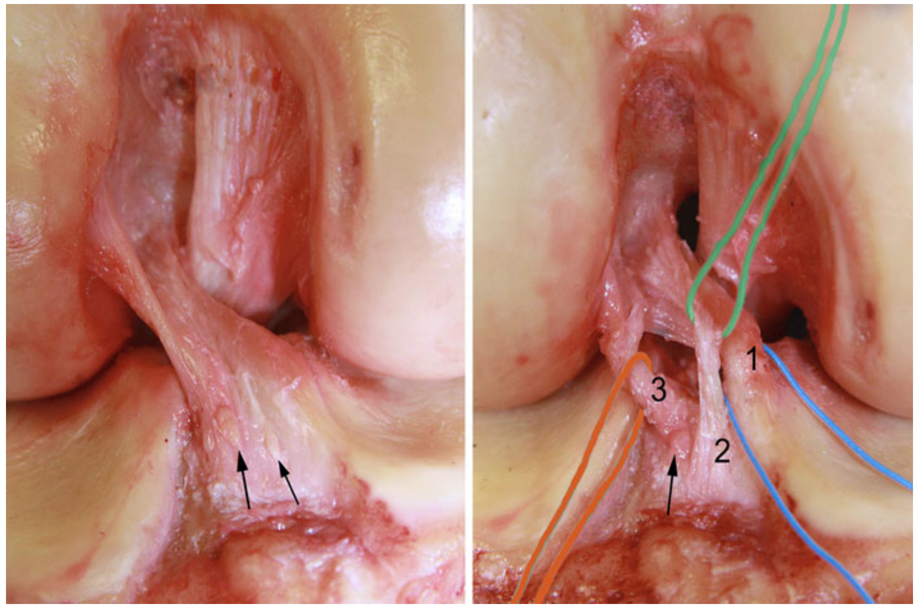

Fig. 8 Human knee (AM-1, IM-2 and PL-3): *Arrows* point to fat protrusion at the gap between AM and IM and IM and PL

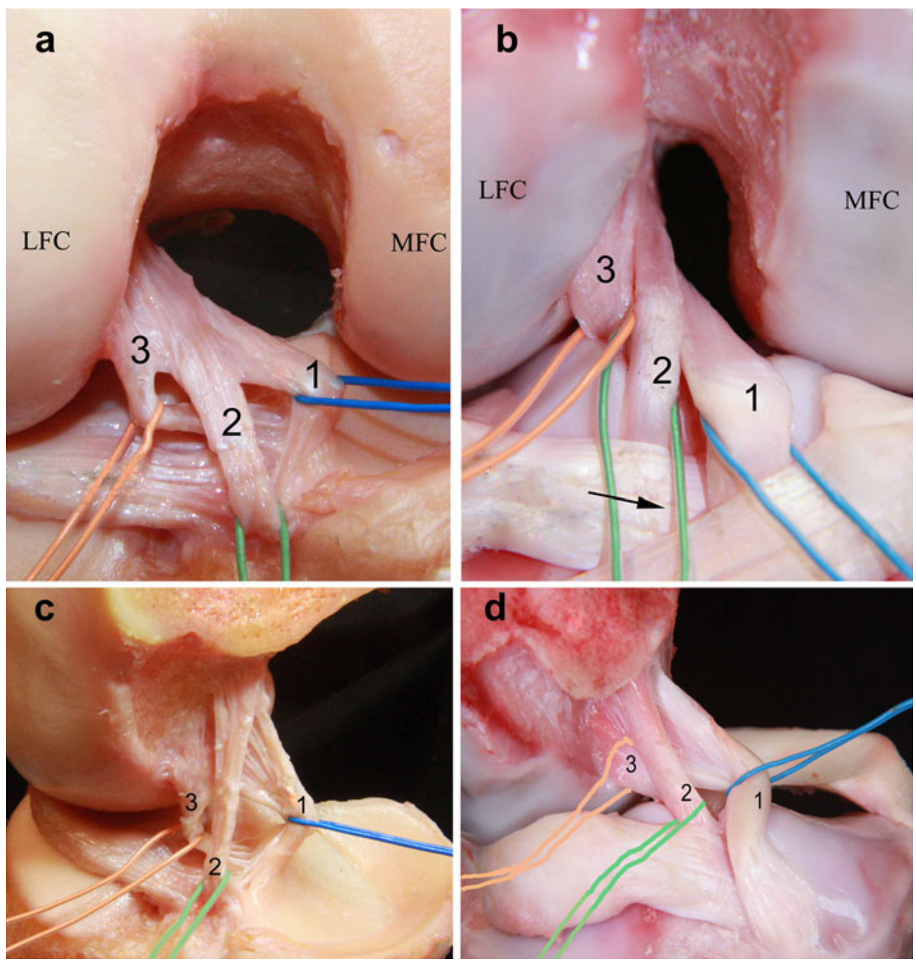

Fig. 9 Comparative Anterior view at 90° flexion with bundles separated (AM-1, IM-2 and PL-3): **a** human ACL and **b** porcine ACL. Medial condyle cut view: **c** human ACL and **d** porcine ACL. *Black arrow* points to the anterior root of the lateral meniscus that passes through a gap between the AM and IM bundle

varied depending on the arrangement of the IM and PL bundles. The AM bundle showed a more consistent arrangement in four out of the five human specimens, and the PL bundle was located posterior to the IM bundle. In one of the specimens, the PL bundle was posterior to IM bundle, but with some extension to the anterior part of

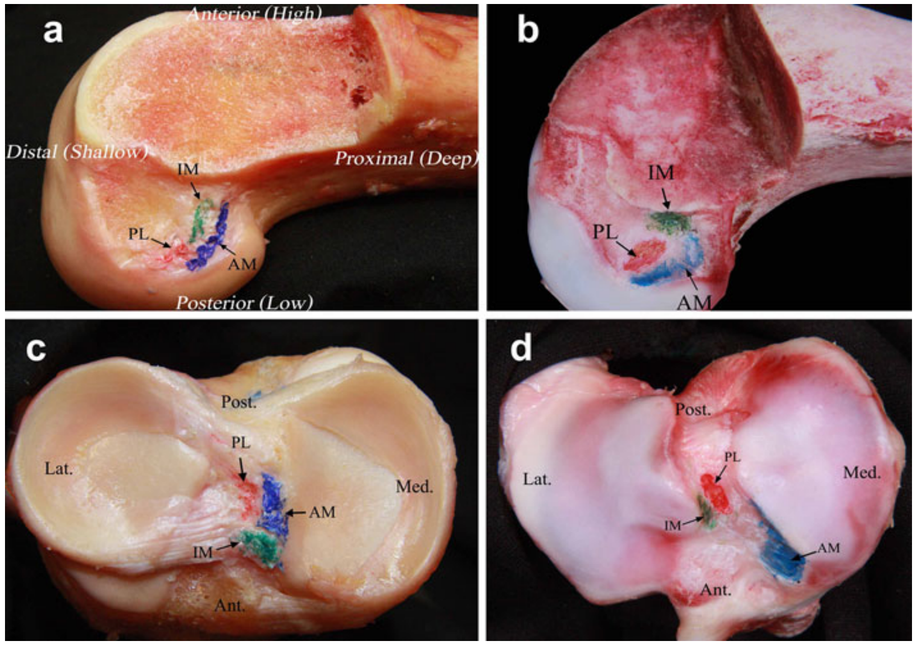

Fig. 10 ACL Comparison views (Blue-AM, Green-IM and Red-PL): **a** human femoral insertion site, **b** porcine femoral insertion site, **c** human tibial insertion site and **d** porcine tibial insertion site

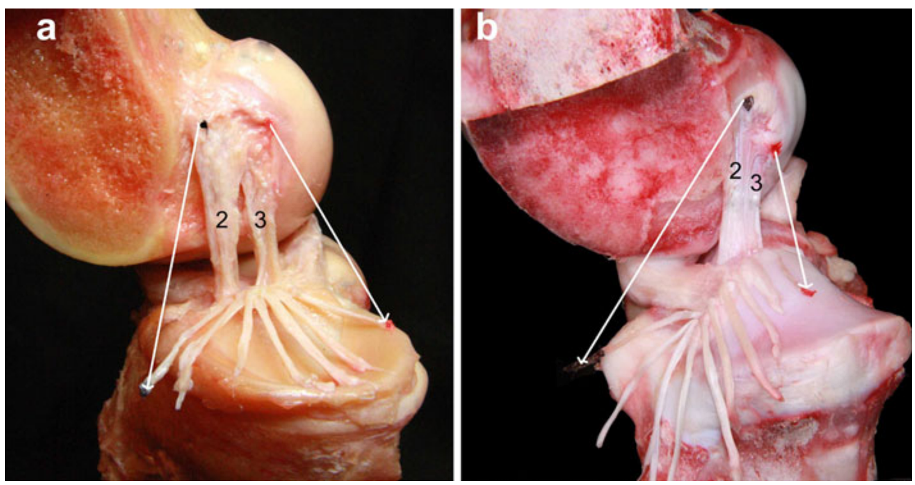

Fig. 11 Specific bundle pattern comparison views: **a** human ACL and **b** porcine ACL. The AM bundle is segmented into 2 mm segments, leaving just the IM (2) and PL (3) attached. In both views the AM segments starting from the most proximal portion (*black dot* on femur) of the insertion site, sequentially tracks towards the anterior aspect of the tibial insertion until the most distal portion (*red dot* on femur) tracks towards the posterior aspect of the tibial insertion site

tibial insertion, lateral to the IM bundle (Fig. 8). However, the main fibres of the PL bundle were still located posterior to the IM bundle.

After using the bundle segmentation technique to explore the ACL in the porcine and human, it was noted that the pattern of femoral and tibial insertion sites were similar: For the human AM bundle, the most proximal portion of the femoral insertion site tracks to the most anterior part of the tibial insertion and the most distal part of femoral insertion tracks to the most posterior part of the tibial insertion (Fig. 11).

The IM and the PL bundles are oriented in an antero-posterior oblique direction at the tibia. Starting at the most proximal part of the femoral insertion site, the 2 mm diameter segments tracked to the most AM part of the tibial insertion site, and the most distal part of the femoral insertion site tracked to the most PL part of the tibial insertion site (Fig. 12).

Discussion

The most important finding of the present study was that the human ACL appears to have three distinct bundles and an attachment pattern similar to the porcine and other animal species. As noted in the literature, the number of bundles varies from one for ten, and even though the ACL is widely accepted as having two functional bundles, there are no ACL studies that explore the ACL solely by its anatomy and not by its function.

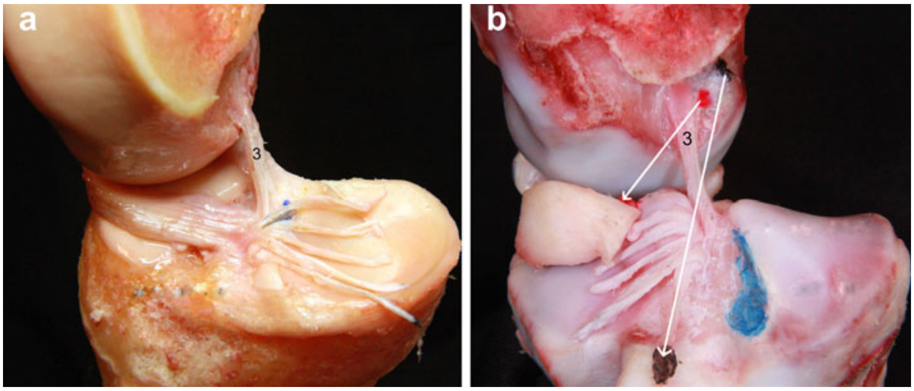

Fig. 12 Specific bundle pattern comparison views: **a** human ACL and **b** porcine ACL. In both views, the AM is cut away and the IM bundle has been segmented away leaving just the PL (3) attached. The IM bundle segments start at the most proximal portion (*black dot* on femur) of the insertion site and sequentially tracks towards the anterior-medial aspect of the tibial insertion until the most distal portion (*red dot* on femur) tracks towards the posterior-lateral aspect of the tibial insertion site

A few studies have examined the human ACL anatomy by cutting and histological assessment to look for a septum between the bundles. Odensen and Gillquist [22] found the human ACL to have only one bundle. This study could not find a clear septum in a cross-sectional view and therefore lacked evidence of separate bundles microscopically. Ferretti et al. [9] histologically examined forty human foetus ACLs and found a vascularized septum between AM and PL, and in two cases, there was another septum in the AM bundle. This finding provides evidence for the hypothesis that human ACL has three bundles. However, this study was performed on human foetuses, not on adult knees.

In 1979, Norwood and Cross [21] first described the ACL as having three bundles: the AM, IM and PL. In Norwood's pictorial description of the bundles, the drawing of the ACL insertion site was attached to the femur in a linear pattern. The bundle located in between the other two was termed the intermediate bundle. The tibial insertion was shown to be triangular in shape with the vertex pointing to the posterior portion of the knee and the IM bundle located lateral to AM bundle. They did not show in detail any landmarks separating the bundles. Amis and Dawkins [3] reported changes in length of three ACL fibre bundles through the range of motion and also showed three bundles with photographs of their dissection. The ACL insertion site at the tibia was different from that of Norwood, which was located in-line, at the tibia and femur; however, the dissecting photos lacked detail of any landmarks separating the bundles.

Recently, Otsubo et al. [23] studied the arrangement and attachment areas of the three bundles of the ACL. They described and showed and how they could separate each bundle. The PL bundle was functionally separated first by careful observations at 90° flexion according to previous studies [2, 4, 5, 7, 11, 16, 24, 25, 33–36]. Then, a gap or septum defining the medial and lateral bundles was found representing the separation between the AM and IM bundles. In this study, the authors used the first functional dissecting technique to divide the PL out of the AM and IM and then divided the AM out of the IM by anatomy. They found the ACL attachment patterns at the femoral insertion site differed from previous studies [3, 10, 14, 21] by noting the PL is located in the posterior-distal half of the femoral insertion site, and the AM is located posterior to the IM at the anterior-proximal half. The tibial insertion sites were similar to the Norwood and Cross' findings.

This study demonstrates a method for separating each bundle in a step-by-step fashion. More importantly, the way each bundle was divided following the fibre orientation, gap and/or fat is based on anatomy, not dependent on function, as in previous studies [1, 2, 4, 5, 7, 11, 16, 23–25, 27, 33–36]. The difference in the approach of the exploration of the ACL was the meticulous removal of all synovial and soft tissue around ACL and to clearly expose and identify each bundle's fibre orientation. The blunt dissecting technique with a Penfield elevator and coloured wires helped to avoid creating a false track while separating the ACL bundles.

In addition to the dissection technique, extensive photography of each specimen was done to document a comparative view for analysis. Also, the fibres of each bundle were separated into 2 mm diameter segments and then cut sequentially at the femur from proximal to distal and tracked carefully in order to establish the specific pattern of insertion site. This has not been done in any previous anatomical study of the ACL. It was found that all specimens have three different fibre orientations of the bundles, and general pattern of the ACL insertion sites are somewhat similar among the different species despite the difference in bony morphology. All the specimens, including human, appeared to have three distinct bundles for the ACL. Surprisingly, the femoral attachment was consistent among all species where the IM bundle stays in-line with PL bundle, right-posterior to the intercondylar ridge and anterior to AM bundle. But, the tibial attachment varied in shape and size [26] depended on the arrangement of IM and PL bundles. The AM bundle was consistent in location being attached along the articular cartilage edge of the medial tibial plateau and the medial intercondylar tubercle in antero-posterior direction (Fig. 4).

In the human ACL specimens, it was found that the ACL is ribbon-like shape, which is consistent with how it was described by Mochizuki et al. [19] and Tena-Arregui et al. [30]. This characteristic was found to be consistent and reproducible among the specimens. In all specimens, a small gap between the AM and the IM could be found, but no macroscopic septum was demonstrated, which is consistent with what was observed by Otsubo et al. [23].

At the femoral insertion site, there was no ACL fibre attachment anterior to the intercondylar ridge [8, 23]. The attachment of each bundle was flat. Only the AM bundle expanded to the rim of posterior condyle cartilage, making its shape like a segment of circle with its anterior border straight and its posterior border convex [5, 8, 11]. This was a consistent finding in all human specimens' dissections. The attachment of each bundle in the tibia fans out and forms a so-called foot region [5, 25]. The shape and size varied but were consistent with many previous studies [6, 13, 17, 28, 29]. Only the AM bundle was consistent in location being attached along the articular cartilage edge of the medial tibial plateau and the medial intercondylar tubercle in antero-posterior direction. The shape and size of tibial insertion depended on the arrangement of IM and PL bundles. One of the five specimens was found to have fatty tissue protrude out between the bundles and had the ACL axis in a medial-lateral direction instead of the expected AM-PL direction (Fig. 8).

The femoral insertion site had a consistent attachment shape. Siebold et al. [28], Colombet et al. [6], Harner et al. [13] and Tallay et al. [29] also found that the ACL tibial insertion site axis was often not in the AM-PL direction. This study found that the tibial insertion site was slightly larger than the femoral insertion site, which follows the findings of Harner et al. [13]. Interestingly, it was found from a comparison of the species that while the femoral insertion sites had a similar outline, the tibial insertion sites varied in shape and size. The ACL is composed of three ribbon-like overlapping bundles. When the knee is extend, the ACL is flat along the lateral wall of the notch, and when the knee flexed, the ACL twists which contracts the mid substance to avoid PCL impingement (twist mechanism), (Fig. 5 in porcine, Fig. 8 in human). From the front view at 90° of knee flexion, the AM bundle always twists and disappears on the path to the femur when it comes closer to posterior cruciate ligament. This was consistent with the Harner et al. [13] study that the mid substance is smaller than insertions.

Limitations of this study were the small number of the human specimens compared to some anatomy studies. In addition, the age of the human knee specimens dissected was older than patients who typically undergo ACL reconstruction. All the specimens except human and porcine were not separated into 2 mm diameter segments to establish the specific pattern of insertion site, because the ACL and the insertion sites had to be preserved for a different study. Lastly, biomechanics of the ACL bundles were not evaluated in this study.

Conclusion

In all species, including human, the ACL had three distinct bundles, the AM, IM and PL. The bundles were composed of multiple fascicles arranged in a definite order and similar among the different species.

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