Fibrocartilage in the transverse ligament of the human acetabulum

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ABSTRACT

Biomechanical experiments on isolated hip joints have suggested that the transverse ligament acts as a bridle for the lunate articular surface of the acetabulum during load bearing, but there are inherent limitations in such studies because the specimens are fixed artificially to testing devices and there are no modifying influences of muscle pull. Further evidence is thus needed to substantiate the theory. Here we argue that if the horns of the lunate surface are forced apart under load, the ligament would straighten and become compressed against the femoral head. It would thus be expected to share some of the features of tendons and ligaments that wrap around bony pulleys and yet previous work has suggested that the transverse ligament is purely fibrous. Transverse ligaments were removed from 8 cadavers (aged 17–39 y) and fixed in 90% methanol. Cryosections were immunolabelled with antibodies against collagens (types I, II, III, VI), glycosaminoglycans (chondroitins 4 and 6 sulphate, dermatan sulphate, keratan sulphate) and proteoglycans (aggrecan, link protein, versican, tenascin). A small sesamoid fibrocartilage was consistently present in the centre of each transverse ligament, near its inner surface at the site where it faced the femoral head. Additionally, a more prominent enthesis fibrocartilage was found at both bony attachments. All fibrocartilage regions, in at least some specimens, labelled for type II collagen, chondroitin 6 sulphate, aggrecan and link protein, molecules more typically associated with articular cartilage. The results suggest that the ligament should be classed as containing a 'moderately cartilaginous' sesamoid fibrocartilage, adapted to withstanding compression. This supports the inferences that can be drawn from previous biomechanical studies. We cannot give any quantitative estimate of the levels of compression experienced. All that can be said is that the ligament occupies an intermediate position in the spectrum of fibrocartilaginous tissues. It is more cartilaginous than some wrap-around tendons at the wrist, but less cartilaginous than certain other wrap-around ligaments, e.g. the transverse ligament of the atlas.

Key words: Entheses; collagens; glycosaminoglycans; proteoglycans; aggrecan.

INTRODUCTION

The transverse ligament is a short fibrous band that bridges across the acetabular notch and is continuous with the fibrocartilaginous labrum that deepens the socket of the hip joint (Williams et al. 1995). Biomechanical experiments suggest that an important function of the ligament is to act as a bridle for the horns of the lunate articular surface of the acetabulum during load bearing, limiting the degree to which the horns can move apart (Lazennec et al. 1997, Vandenbussche et al. 1999). When loads of 2800 N were applied to isolated hip joints with intact transverse ligaments in material testing devices, the acetabular notch widened by 3.2% (Löhe et al. 1994) or 43 μm (average value; Vandenbussche et al. 1999). When the transverse ligament is cut, the mobility of the acetabular horns is higher than it is with an intact ligament even under loads of only 1000 N (Lazennec et al. 1997). Any widening of the notch will necessarily straighten the ligament and thus increase the compressive force exerted on it by the femoral head.

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Table 1. *Primary antibodies used for immunohistochemistry**

Antigen(s) recognised	Antibody	Dilution	Host	Enzyme pretreatment	Source	Reference
Collagen I	Col 1	1:2000	Mouse	Hyal (1.5 IU/ml) & ChABC (0.25 IU/ml)	Sigma	
Collagen II	CIICI	1:5	Mouse	Hyal (1.5 IU/ml) & ChABC (0.25 IU/ml)	DSHB	Holmdahl et al. (1986)
Collagen III	4H12	1:500	Mouse	Hyal (1.5 IU/ml) & ChABC (0.25 IU/ml)		
Collagen VI	5C6	1:4	Mouse	Hyal (1.5 IU/ml) & ChABC (0.25 IU/ml)	DSHB	Hessle & Engvall (1984)
Chondroitin-4-sulphate	2B6	1:1500	Mouse	ChAC (0.25 IU/ml)	B. Caterson	Caterson et al. (1985)
Chondroitin-4 & dermatan sulphates	2B6	1:1500	Mouse	ChABC (0.25 IU/ml)	B. Caterson	Caterson et al. (1985)
Chondroitin-6-sulphate	3B3	1:80	Mouse	ChABC (0.25 IU/ml)	B. Caterson	Caterson et al. (1985)
Keratan sulphate	5D4	1:1500	Mouse	None	B. Caterson	Caterson et al. (1983)
Aggrecan	1C6	1:100	Mouse	ChAC (0.25 IU/ml) after reduction & alkylation	B. Caterson	Calabro et al. (1992)
Link protein	8A4	1:100	Mouse	ChAC (0.25 IU/ml) after reduction & alkylation	B. Caterson	
Versican	12C5	1:10	Mouse	ChAC (0.25 IU/ml)	B. Caterson	
Tenascin	T2H5	1:100	Mouse	ChAC (0.25 IU/ml)		

* All are monoclonal antibodies. All enzyme pretreatments were carried out at 37 °C in 0.1 M tris-acetate buffer at pH 7.8 for 30 min. Ch, chondroitinase; Hyal, hyaluronidase.

Further compression could result from incongruity between the femoral head and the acetabular socket that is confirmed by variations in the width of the joint space (Eckstein et al. 1997; von Eisenhart-Rothe et al. 1997).

All these experiments, however, have been conducted on isolated hip joints where the biomechanical conditions differ substantially from those that occur in vivo during standing and walking. The biomechanical differences relate not only to the manner in which the specimens are fixed to the testing device, but also to the absence of any muscle pull. Due to such inherent difficulties in simulating the normal biomechanical situation in tests on isolated specimens, we cannot therefore be sure whether the transverse ligament is really subject to compression in vivo.

The purpose of the present investigation is to approach the problem from a very different angle. We argue that if the transverse ligament is subject to intermittent compression because the horns of the lunate surface move apart on loading and because the joint is incongruous, it would be expected to share some of the features of tendons and ligaments that wrap around bony pulleys. Wrap-around tendons include those that press against the malleoli (Benjamin et al. 1995; Benjamin & Ralphs, 1995, 1997, 1998; Malaviya et al. 2000) and wrap-around ligaments include the transverse ligament that holds the dens in contact with the atlas (Stofft, 1968; Saldinger et al. 1990; Milz et al., unpublished observations). They can be characterised by a sesamoid fibrocartilage in which cartilage-like cells and cartilage-like extracellular matrix (ECM) are present (Vogel, 1995; Berenson et al. 1996; Waggett et al. 1998). Such an ECM frequently contains aggrecan and type II collagen.

MATERIALS AND METHODS

Transverse acetabular ligaments were removed from 8 human cadavers (both sexes; ages 17–39 y) within 36 h of death and fixed for 24 h in 90% methanol at 4 °C. The specimens were stored as necessary at −20 °C. In all bodies, the whole ligament was removed (i.e. from one enthesis to the other) and the specimens decalcified in 5% EDTA. The samples were then rinsed in phosphate buffered saline (PBS), infiltrated overnight with 5% sucrose in PBS and cryosectioned at 12 μm. Sections were stained with toluidine blue in order to highlight the presence of any fibrocartilage by its metachromasia, and with a panel of monoclonal and polyclonal antibodies (Table 1). The antibodies were directed against collagens (types I, II, III, VI), GAGs (chondroitin-4 and -6-sulphates, dermatan sulphate and keratan sulphate) and proteoglycans (aggrecan, link protein, versican and tenascin). Sections immunolabelled for aggrecan and link protein were treated with 10 mM dl-dithiothreitol (Sigma) in 50 mM Tris HCl, 200 mM sodium chloride, pH 7.4, for 2 h at 37 °C and then alkylated with 40 mM iodoacetamide in PBS for 1 h at 37 °C. The sections

were subsequently incubated at 37 °C with chondroitinase AC (0.25 units per ml; Sigma). Endogenous peroxidase activity was blocked in all sections by pretreatment with 0.3% hydrogen peroxide in methanol for 30 min and nonspecific binding of the secondary antibody was reduced with an appropriate serum block for 40 min. We controlled for nonspecific binding of antibodies by omitting the primary antibody, or by incubating the sections with normal mouse immunoglobulins (10 μg/ml for all monoclonal antibodies). Antibody binding was detected with a Vectastain ABC 'Elite' avidin/biotin/peroxidase kit.

RESULTS

A small layer of fibrocartilage cells that constituted a sesamoid fibrocartilage was consistently present in the centre of each transverse ligament, near its inner surface at the site where it faced the femoral head (Fig. 1*a*). Additionally, a more prominent enthesis fibrocartilage was found at both bony attachments (Fig. 1*b–d*). Other regions were fibrous and the distribution of all the tissues is shown diagrammatically in Figure 2. Although the size of the sesamoid fibrocartilage varied from specimen to specimen, it was always characterised by cartilage-like cells scattered in a metachromatic ECM. Its immunolabelling characteristics, together with those of the rest of the transverse ligament, are summarised in Table 2.

Collagens

Types I, III and VI collagens were found in all regions of the ligament (Fig. 1*c*, *e–g*), though type I collagen labelling was locally absent at small parts of the enthesis (Fig. 1*f*). Although there was no difference in the labelling intensity for type VI collagen in the different parts of the ligament, labelling was more pericellular in the sesamoid and enthesis fibrocartilages than elsewhere. Pericellular labelling was particularly prominent near the articular surface (Fig. 1*g*).

Extensive labelling for type II collagen was characteristic of both enthesis fibrocartilages in all transverse ligaments (Fig. 1*b*, *d*); there was no difference between one end of the ligament and the other. Type II collagen was seen in the sesamoid fibrocartilage in 3 of the 8 specimens (Fig. 1*a*).

Glycosaminoglycans and proteoglycans

Keratan, dermatan and chondroitin-4-sulphates were found in all regions of the ligament (Fig. 1*h*, *i*), but chondroitin-6-sulphate was only found in the enthesis and sesamoid fibrocartilages, where its labelling intensity varied from moderate to strong (Fig. 1*j*, *k*). Staining of chondroitin-6-sulphate in the enthesis fibrocartilages was largely pericellular and highlighted the interwoven arrangement of fibre bundles that characterised this region (Fig. 1*k*).

Labelling for versican was predominantly seen in the fibrous regions of the ligament. In contrast, aggrecan and link protein were only detected in the fibrocartilaginous regions (Fig. 1*l*, *m*). Labelling for aggrecan and link protein was generally uniformly distributed throughout the ECM, but in some fibrocartilages, labelling was pericellular and reminiscent of that of chondroitin-6-sulphate.

DISCUSSION

The results suggest that the central part of the transverse acetabular ligament should be classed as a moderately 'cartilaginous' sesamoid fibrocartilage. Our histological and immunohistochemical data are thus consistent with the biomechanical analyses of Löhe et al. (1994, 1996), Lazennec et al. (1997) and Vandenbussche et al. (1999) that suggest the ligament is subject to some compression during load bearing of the hip joint, because the horns of the lunate surface move apart and/or because the joint is slightly incongruous. However, we cannot give any quantitative estimate of the levels of compression experienced. All we can say is that in the broad spectrum of ligament/tendon tissues that range from fibrous to cartilaginous, the transverse ligament occupies an intermediate position. It is more fibrous than some, e.g. the ligament that holds the dens in contact with the atlas (Milz et al., unpublished observations), but more cartilaginous than others, e.g. the tendon of extensor pollicis longus that turns around the dorsal tubercle of the radius (Benjamin et al. 1995). The ligament has a small number of strategically-located fibrocartilage cells on its inner surface nearest the site of contact with the femoral head—contrary to earlier statements (Williams et al. 1995). Furthermore, at least some specimens label weakly for aggrecan and type II collagen, molecules typical of articular cartilage that are associated with its compression-tolerance properties. In articular cartilage, the high charge density of the sulphated glycosaminoglycans in aggrecan attracts water into the tissue. The aggrecan and water are then held in place by the fibrous network of type II collagen molecules.

In addition to the sesamoid fibrocartilage in the middle of the transverse acetabular ligament, we also

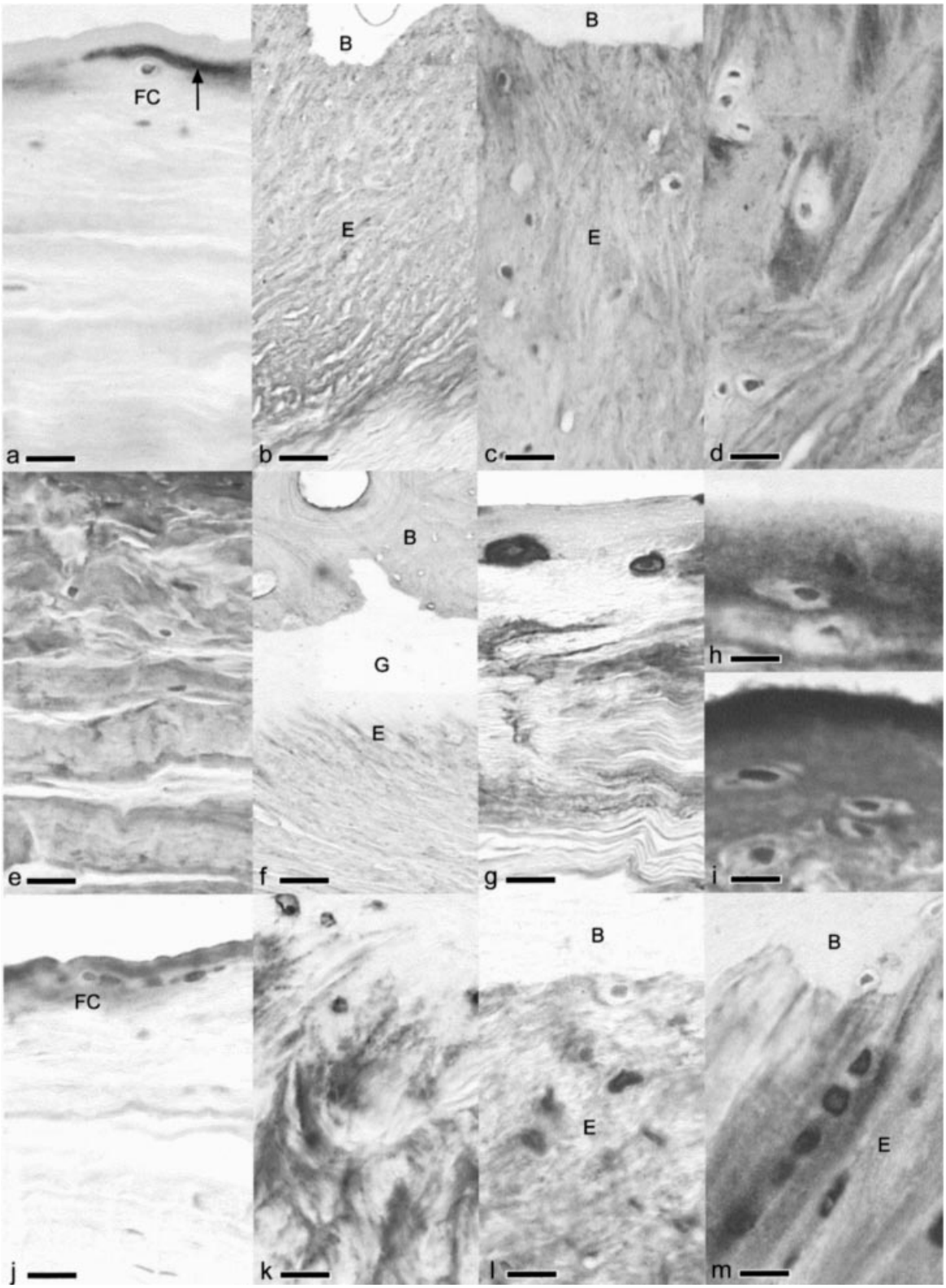

Fig. 1. (*a*) High power view of the sesamoid fibrocartilage showing large fibrocartilage cells (FC) lying just beneath the inner surface of the ligament. Immunohistochemical labelling for type II collagen shows a thin but distinct region of staining (arrow). Bar, 20 μm. (*b*) Low power view of type II collagen labelling at an enthesis (E). B, bone. Bar, 80 μm. (*c*) Strong, pericellular labelling for type III collagen in an enthesis fibrocartilage. B, bone; E, enthesis. Bar, 20 μm. (*d*) High power view of a prominent enthesis fibrocartilage with strong pericellular labelling for type II collagen. Bar, 20 μm. (*e*) Widespread diffuse labelling for type III collagen in a sesamoid fibrocartilage. Bar, 20 μm. (*f*) Enthesis labelling for type I collagen. Note the nonlabelled gap (G) between the bone (B) and the rest of the enthesis fibrocartilage (E). Bar, 80 μm. (*g*) Patchy pericellular labelling for type VI collagen in the sesamoid fibrocartilage. Bar, 20 μm. (*h*,*i*) Labelling for chondroitin-4 and dermatan sulphates with antibody 2B6 + chondroitinase ABC (*i*), and labelling for chondroitin-4-sulphate alone, with antibody 2B6 + chondroitinase

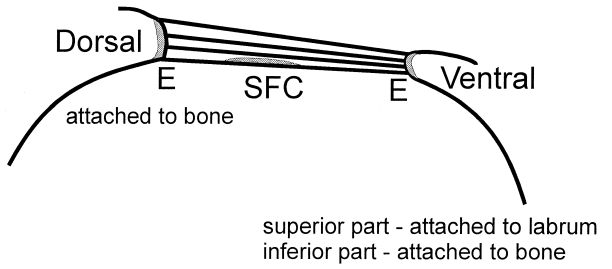

Fig. 2. Schematic drawing of the transverse ligament to show its morphological regions: enthesis fibrocartilage (E) at the attachments of the ligament to the ventral and posterior horn of the lunate surface, the intervening fibrous region of the ligament and the sesamoid fibrocartilage (SFC). Note that the femoral head presses against the inner surface of the ligament where the sesamoid fibrocartilage is situated.

found that there are conspicuous enthesis fibrocartilages at its bony attachments. This is interesting because this ligament only connects one part of a bone to another and does not link different bones across a joint like most ligaments. Consequently, joint movements that lead to the bending of collagen fibres in the ligament near an enthesis (and which could lead to stress concentration at the bony interface; for review see Benjamin & Ralphs, 1995) cannot operate in this case. Nevertheless, some minimal bending of the transverse ligament can occur as it straightens under tension. At other sites in the body however, when a joint moves, there can be very substantial bending of the tendon/ligament fibres relative to the bone that could alone account for the presence of a prominent enthesis fibrocartilage (Benjamin et al. 1986; Lewis et al. 1998). The greater the degree of bending (and thus the local compression), the more fibrocartilage is present (Evans et al. 1990; Benjamin & Ralphs, 1995). More important however in accounting for the presence of a conspicuous enthesis fibrocartilage within the transverse ligament, may be the increase in shear stress that accompanies increased tension when the ligament is straightened. Although the levels of tension in the ligament are unknown, they are likely to be quite large because of the change in length that occurs in the ligament during loading (Löhe et al. 1994, 1996; Lazennec et al. 1997; Vandenbussche et al. 1999). Biologically relevant levels of shear at the interface between soft and hard tissue could result from a high increase in the levels of tension and be the mechanical stimulus responsible for triggering the development of enthesis fibrocartilage. Finally, it is also possible that the fibrocartilage is prominent because of the length changes that occur in the transverse ligament during weight bearing and gait (Löhe et al. 1994, 1996; Lazennec et al. 1997; Vandenbussche et al. 1999). When any ligament lengthens, it must inevitably narrow and this must not be allowed to occur too close to the calcified interface —for this would increase stress concentration. This 'stretching-brake' theory of enthesis fibrocartilage function was originally suggested by Knese & Biermann (1958), but has received little subsequent attention. The finding of aggrecan and link protein in the entheses of the transverse ligament and elsewhere (Waggett et al. 1998) could explain why entheses do not allow narrowing to occur at this site during ligament stretching. Aggrecan imparts resistance to compression by virtue of the water it attracts, and if a ligament cannot be compressed in a particular region, it also cannot be stretched there either.

Table 2. *Summary of the immunolabelling characteristics of the different regions of the transverse ligament**

	ECM molecule								
Region	Type II Collagen	KS	DS, C4S	C4S	C6S	Aggrecan	Link protein	Versican	Tenascin
Ventral enthesis	7	8	8	7	7	5	5	5	5
Dorsal enthesis	8	8	8	7	8	7	8	1	5
Inner part of ligament (sesamoid fibrocartilage)	3	8	8	7	6	4	4	6	7
Outer part of ligament	0	8	8	6	0	1	0	7	7

* Each column entry shows the number out of the total of 8 ligaments examined, in which positive labelling was identified. C6S, chondroitin-6-sulphate; C4S, chondroitin-4-sulphate; DS, dermatan sulphate; KS, keratan sulphate.

AC (*h*) in the sesamoid fibrocartilage. The weaker labelling in (*h*) indicates that much of the labelling in (*i*) is associated with dermatan sulphate. Bar, 10 μm in both. (*j*) Distinct pericellular labelling for chondroitin-6-sulphate at the inner surface of the transverse ligament. Note the presence of sesamoid fibrocartilage cells (FC). Bar, 20 μm (*k*) Labelling for chondroitin-6-sulphate in the enthesis fibrocartilages is largely pericellular and highlights the interwoven arrangement of fibre bundles that characterise this region. Bar, 20 μm. (*l–m*) Enthesis fibrocartilage (E) labelling for aggrecan (*l*) and link protein (*m*). B, bone. Bar, 20 μm for both.

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