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The Spine Journal 15 (2015) 1325–1331

THE SPINE JOURNAL

Basic Science

The molecular composition of the extracellular matrix of the human iliolumbar ligament

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Received 5 April 2012; revised 12 June 2013; accepted 25 July 2013

Abstract

BACKGROUND CONTEXT: The human iliolumbar ligament connects the transverse process of L5 to the iliac crest and contributes to lumbosacral stability and has been associated with low back pain. However, different opinions exist regarding the functional relevance of the ligament.

PURPOSE: In the present study, we analyze the regional molecular composition of the ligament extracellular matrix.

STUDY DESIGN: Special attention is given to the attachment sites, to determine whether the ligament is subjected to a certain mechanical environment.

METHODS: Iliolumbar ligament samples, extending from one enthesis to the other, were removed from 11 cadavers and fixed in methanol. Cryosections were immunolabeled with a panel of antibodies directed against collagens, glycosaminoglycans, proteoglycans, matrix proteins, and neurofilament.

RESULTS: The mid-substance of the ligament labeled for all the molecules normally found in dense fibrous connective tissue including types I, III, and VI collagen, versican, dermatan -, chondroitin 4 -, and keratan sulfate. However, both entheses were fibrocartilaginous and labeled for type II collagen, aggrecan, and chondroitin 6- sulfate. A common feature was fat between the fiber bundles near the entheses. Occasionally this fat contained nerve fibers.

CONCLUSIONS: The existence of fibrocartilaginous entheses suggests that the insertion sites of the ligament are subject to both tensile and compressive loading—probably because of insertional angle changes between ligament and bone during loading. Our findings support the suggestion that the iliolumbar ligament might play an important role in the stabilization of the lumbosacral junction. © 2015 Elsevier Inc. All rights reserved.

Keywords: Insertion; Enthesis; Attachment; Type II collagen; Lumbosacral junction

Introduction

The iliolumbar ligament connects the transverse process of L5 to the iliac crest and contributes to the stabilization of the lumbosacral junction. This is of clinical interest as the lumbosacral junction is prone to pathologies that affect spine function. Interestingly, the detailed anatomical reports

FDA device/drug status: Not applicable.

Author disclosures: ***AFV:*** Nothing to disclose. ***YS:*** Nothing to disclose. ***CMS:*** Nothing to disclose. ***BMB:*** Research Support (Investigator Salary, Staff/Materials): Aesculap (B), Alphatech (B), Macromed (B); Grants: Synthes (D); Fellowship Support: Medtronic (D). ***AB:*** Nothing to disclose. ***MB:*** Nothing to disclose. ***SM:*** Nothing to disclose.

The disclosure key can be found on the Table of Contents and at www.TheSpineJournalOnline.com.

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http://dx.doi.org/10.1016/j.spinee.2013.07.483

regarding the course of the ligament vary. Early descriptions of the ligament [1,2] state that it is not only constituted by fibers from L5 to the iliac crest but by a variable number of fiber bundles that reach out in various directions. Currently, the ligament is classified as a single- or multi-stranded structure that connects the transverse process of L5 with the iliac crest [3] or iliac tuberosity [4]. Various authors [5–7] also include fiber bundles originating from the transverse process of L4 and others [8,9] add fiber bundles running toward the sacrum.

Biomechanical experiments on the function of the iliolumbar ligament [10–14] are usually conducted using cadaveric material and thus the extent to which they can assess in vivo function is limited. However, overload on the ligament, especially on its attachment at the iliac crest, is presumed to cause low back pain [15–18]. Some authors believe that this pain syndrome (iliolumbar syndrome) is responsible for up to 50% of all low back pain cases [19,20]. It has been postulated that pain is perceived by a neuronal network comparable with that found in other ligaments [18]. Recently, mechanoreceptors and free nerve endings have been reported in the ligament near its attachment sites and the function of the ligament to act primarily as a biomechanical stabilizer has been questioned [21].

Previous work has shown that tendons and ligaments have the ability to adapt to mechanical stress and that such an adaptation is reflected by the local composition of the extracellular matrix (ECM) [22,23]. Briefly, a fibrocartilaginous enthesis indicates that the attachment site is not only subject to tensile but also compressive or shear forces. At such sites, fibrocartilage cells produce an ECM that allows the tissue to bear compressive stress and dissipate the stress away from the hard-soft tissue interface. In many regions of the musculoskeletal system, it has been shown that ‘‘insertional angle change’’ is one key factor that contributes to the expression of fibrocartilage at entheses. From a biomechanical standpoint, it is expected that the insertional angle changes would occur at either enthesis of the ligament if it is engaged in its function as a stabilizing structure during motion across the lumbopelvic junction.

Therefore, the present study aimed to determine the regional composition of ECM of the human iliolumbar ligament and its attachment sites to clarify whether the molecular distribution pattern corresponds to that of other mechanically loaded ligaments.

Materials and methods

Bone-ligament-bone complexes, extending from the enthesis at the transverse process of L5 to the attachment at the iliac crest, were removed within 48 hours of death, from 11 cadavers of both sexes (average age, 34.2 years; age range, 13–48 years; 9 male, 2 female). In each cadaver, the ligament was removed from one side of the body only. Removal was performed according to the ethical regulations of the medical faculty of LMU Munich in the department for legal medicine.

The specimens were fixed for at least 6 hours in 90% methanol at 4°C and then were stored at −20°C in 100% methanol for at least 1 week. Before decalcification in 12.5% ethylenediaminetetraacetic acid, the specimens were trimmed to minimize the amount of bone tissue. The large size of some specimens did not allow sectioning in one piece and these ligaments were separated into two halves. All specimens were cryosectioned parallel to the long axis of the ligament at a thickness of 12 μm and mounted on superfrost plus glass slides (Gerhard Menzel GmbH, Thermo Fisher Scientific, Braunschweig, Germany). A few sections from each sample were stained with toluidine blue.

Immunohistochemical procedure

Labeling was performed with a panel of mono- or polyclonal antibodies against collagens (types I, II, III, VI, X, XII, XIV), glycosaminoglycans (keratan sulfate, dermatan sulfate, chondroitin-4-, and -6-sulfates), proteoglycans (lumican, versican, aggrecan), and matrix proteins (link protein, cartilage oligomeric matrix protein, fibromodulin) and neurofilament. The immunohistochemical protocol is similar to that used in previous studies [22,24] and details concerning antibodies and pretreatment are given in Table 1. Briefly, nonspecific binding of the antibodies was blocked using horse serum, and control sections were obtained by omitting the primary antibody. The Vectastain ABC ‘‘Elite’’ avidin/biotin kit (Vector Labs, Burlingame, CA, USA) was used for detection of the primary antibodies; Mayer’s haematoxylin acted as a nuclear counterstain.

Results

The iliolumbar ligament could be defined anatomically in all donors. In our specimens, the overwhelming majority of its fibers originated from the tip of the transverse process of L5 and inserted into the superior and dorsal aspect of the iliac crest. Occasionally, two separate anterior and posterior strands of the ligament were clearly distinguished during preparation (Fig. 1). In these cases, large amounts of fat filled the gap between the two parts of the ligament (epitenon fat, Fig. 1, Top Right). Adipose tissue was often found between the ligament fiber bundles in the mid-substance and sometimes near the enthesis (endotenon fat, Fig. 2, B).

Immunohistochemistry

Both attachment sites of the iliolumbar ligament showed all the characteristic features of fibrocartilaginous entheses and labeled positively for cartilage-related molecules (Fig. 2; Table 2). Among these were type II collagen, chondroitin-6-sulfate, aggrecan, and link protein. Although quantitative assessment of the region labeling positively for these molecules was not performed, an obvious difference in the size of the positive region could be found. In certain individuals, collagen II labeling was more prominent at the iliac crest attachment than at the transverse process enthesis (Figs. 2, A and B). In the mid-substance of the ligament,

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