The Peripheral Neuronal Phenotype is Important in the Pathogenesis of Painful Human Tendinopathy: A Systematic Review

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Abstract

Background The pathogenesis of tendinopathy is complex and incompletely understood. Although significant advances have been made in terms of understanding the pathological changes in both the extracellular matrix and the cells involved, relatively little is known about the role of neuronal regulation in tendinopathy. The frequent mismatch between tendon pathology and pain may be explained, in part, by differences in the peripheral neuronal phenotype of patients.

Questions/purposes The primary purpose of this review was to determine whether evidence exists of changes in the peripheral neuronal phenotype in painful human tendinopathy and, if so, to identify the associated histological and molecular changes. The secondary purpose was to determine if any changes in the peripheral neuronal phenotype reported correlate with pain symptoms.

Methods We conducted a systematic review of the scientific literature using the PRISMA and Cochrane guidelines. The Medline and Embase databases were searched using specific search criteria. Only studies analyzing the peripheral tissue of patients with the clinical

diagnosis of tendinopathy were included. Inclusion was agreed on by two independent researchers on review of abstracts or full text.

Results Overall in the 27 included studies, there was clear evidence of changes in the peripheral neuronal phenotype in painful human tendinopathy. The excitatory glutaminergic system was significantly upregulated in seven studies, there was a significant increase in sensory neuropeptide expression in four studies, and there were significant changes in the molecular morphology of tenocytes, blood vessels, and nerves. In rotator cuff tendinopathy, substance P has been shown to correlate with pain and the neural density in the subacromial bursa has been shown to correlate with rest pain.

Conclusions The peripheral neuronal phenotype is an important factor in the pathogenesis of painful human tendinopathy. Further research in this area specifically correlating tissue changes to clinical scores has great potential in further developing our understanding of the disease process.

Introduction

Painful tendinopathy is a frequently encountered clinical problem, the three most common sites affected being the Achilles, patellar, and rotator cuff tendons [62]. Rotator cuff tendinopathy (RCT) is the most common of all with an annual incidence of over 1% that greatly increases with age [20, 60]. Other tendons affected include those around the elbow (medial and lateral epicondylitis) and around the wrist. There is a significant disease burden attached to painful tendinopathy not only in terms of healthcare costs, but also in terms of time off work and impact on patients' quality of life [44]. Therefore, the effective treatment of painful tendinopathy has a huge potential to save money

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and improve quality of life [38]. In general, the etiology of these tendinopathies remains unclear, although the commonly affected tendons all experience high levels of mechanical stress [48] and overuse is a frequently implicated risk factor [26].

The molecular pathophysiology of tendinopathy has become clearer in recent years [28, 51]. Tendinopathy has characteristic histopathological, clinical, and radiological findings [62]. Clinical symptoms including pain are often poorly matched to the histopathological and radiological findings, meaning that a high proportion of patients with a tendon that is both histopathologically and radiologically abnormal have no pain or other symptoms [22, 27, 66]. The reasons for this mismatch between pathology and perceived pain are poorly understood [27]; however, recent research has identified the peripheral and central pain processing pathways as good candidates for an explanation [32, 35]. It appears that the presence of pain in tendinopathy not only requires mechanical changes in the tendon, but also alterations in the way the local cells and the peripheral nerves react to this change, thus resulting in the nociceptive pathways to higher centers being activated [27]. Some of the mechanisms that drive increased peripheral nerve sensitivity (peripheral sensitization) are depicted (Fig. 1).

The peripheral nervous system plays an important role in tissue homeostasis and tendon healing [2]. The neuronal response to tendon injury involves nerve in-growth during the initial inflammatory phase; the subsequent proliferative and remodeling phases are regulated by sensory nerves as well as the glutaminergic and autonomic systems. Sensory neuropeptide expression has been shown to be associated with both failed healing and pain in an animal model of tendon injury [45]; they have also been causally linked with tendinopathy-like changes in animal models [8, 10]. The application of neuropeptides such as substance P has shown promise in improving tendon healing in an animal model of tendon rupture [19], but this has not been taken further into in vivo human studies. Neuropeptides have pro-proliferative, angiogenic, and stem cell-stimulating properties in vitro [11], therefore implicating them further in potentially aiding tendon healing.

In this context, the primary purpose of this systematic review of the literature was to determine whether there was evidence of changes in the peripheral neuronal phenotype in painful human tendinopathy. The term “peripheral neuronal phenotype” is used in this context to refer to specific characteristics of the peripheral nervous system including the nerves, the neuronal mediators, and the receptors for these mediators in peripheral tissue. We also aimed to describe the relevant histological and molecular changes to the peripheral neuronal phenotype as part of this

primary purpose. A secondary purpose was to determine whether any changes in peripheral neuronal phenotype were correlated to pain symptoms.

Materials and Methods

This systematic review used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement and the Cochrane handbook as guidelines in the development of the study protocol and the report of the current study [21, 43]. The inclusion criteria and methods of analysis were specified in advance and documented in a protocol.

Information Sources and Search Strategy

Studies were identified by searching the Medline (1951 to present) and Embase (1980 to present) electronic databases. The search was undertaken in October 2012. The following search terms were used: tendinopathy AND pain, nerve, neuronal, innervation, somatic, autonomic, sympathetic, parasympathetic, nerve growth factor, NGF, TrkA, p75, neuropeptide*, NPY, adrenaline, noradrenaline, adrenoceptors, VIP, acetylcholine, substance P, NK1, CGRP, calcitonin, RAMP, IB4, galanin, receptor*, nocicept*, mechanorecept*, proprioceptor*, Ruffini, Meissner, golgi, opio*, neurohistolog*, somatostatin, enkephalin, endorphin, neurokinin, NMDA, AMPA, mGluR, glutam*, metabotropic, ionotropic, neurotrophins, Nav1.8, sodium channels, histology, histological.

Additional studies were located by searching reference lists of short-listed articles. The citations identified from the searches were combined and duplicates excluded. All citations for papers clearly referring to a topic other than painful human tendinopathy were excluded as were others whose title clearly showed that the paper was not relevant to the current study. Papers had to relate to peripheral neuronal phenotype, which included innervation, peripheral neuronal mediators, and their receptors, including gene expression. Studies had to be of human participants who had been diagnosed with painful tendinopathy based on a clinician's history and clinical examination findings with or without radiological assessment.

Case reports and review articles were excluded. In vitro studies and animal studies were excluded. Studies relating to patients without pain were excluded as were studies relating to changes after surgical intervention. Studies that did not analyze changes in the peripheral tissue were excluded. Studies that did not use control groups were also excluded. Studies that could not be obtained in English were excluded.

Fig. 1 A schematic diagram depicting mechanisms of peripheral sensitization and inflammation at the peripheral nerve terminal. ASIC = acid sensing ion channel; iGluR = ionotropic glutamate receptor; IL-1 β = interleukin-1 β ; IL-6 = interleukin-6; mGluR = metabotropic glutamate receptor; NGF = nerve growth factor; PGE₂ = prostaglandin E₂; PKA = protein kinase A; PKC = protein kinase C; TNF- α = tumor necrosis factor α ; TrkA = tyrosine kinase receptor A; TTXr = tetrodotoxin-resistant sodium channel; μ = mu-

opioid receptor; M₂ = muscarinic receptor; 5-HT₃ = serotonin; H₁ = histamine receptor; B₁/B₂ = bradykinin receptors; A₂ = adenosine receptor; GABA = γ -aminobutyric acid receptor; EP = prostaglandin receptor; VEGF = vascular endothelial growth factor. Reproduced with permission from Dean BJ, Gwilym SE, Carr AJ. Why does my shoulder hurt? A review of the neuroanatomical and biochemical basis of shoulder pain. *Br J Sports Med.* 2013 Feb 21 [Epub ahead of print] [27].

The search, selection of studies, and data analysis were performed independently by two individuals (BD, SF). Agreement on inclusion was achieved after review of the full-text articles and a joint decision by both individuals based on the inclusion/exclusion criteria. The data were then extracted using a spreadsheet designed by two authors (BD, SF); this included data relating to study heterogeneity and methodological quality. The data extracted included patient demographics, symptom duration, investigations, control group type, tissue analysis method, and statistical methods. Methodological quality was assessed using a

10-point scale based on the scale used by Hegedus et al. [36] (Table 1).

Study Selection

The search strategy revealed a total of 5732 results (Fig. 2). After removal of 1215 duplicate entries, 4517 unique papers remained. Screening of the titles and abstracts revealed 46 papers eligible for inclusion and the hand search of references revealed another 20 papers potentially

Table 1. Methodological quality assessment document (the number of yes answers was counted for each study to give a total score out of 10)

Number	Criteria	Yes	No	Unclear
1	Inclusion/exclusion criteria clearly described			
2	Study population clearly described			
3	Study population representative of those in clinical practice			
4	Control group clearly described			
5	Sampling method clearly described			
6	Live study subjects			
7	Live control subjects			
8	Quantitative method or semiquantitative method using minimum of two independent observers			
9	Reliability and/or validity of methods described			
10	Study limitations addressed

Fig. 2 A flow diagram demonstrating the systematic review protocol.

eligible for inclusion. Further assessment of eligibility, based on full-text papers, led to the exclusion of 39 of these 66 papers. The reasons for the exclusion of these 39 papers were as follows: no control group (three), reviews (17), animal studies (13), or non-neuronal tissue changes (six). This left 27 papers meeting our criteria for inclusion, which are summarized in Appendix 1 [3–7, 9, 12–17, 23–25, 29, 34, 42, 53–56, 58, 59, 61, 64, 68].

Study Characteristics

Of the 27 studies included, 11 related to the Achilles tendon, seven to the patellar tendon, two to both the Achilles and patellar tendons, five to RCT, and two to medial/lateral humeral epicondylitis. All studies related to patients with painful tendinopathy and all studies stated specific diagnostic criteria. The majority of these studies specified that the tendinopathy was of a chronic duration (25 of 27 studies); the remaining two studies did not specify the duration of symptoms. The mean ages of the overall patient groups were as follows: 28 years (patellar), 40 years (medial/lateral epicondylitis), 46 years (Achilles), and 56 years (RCT).

In 23 of the 27 studies, the tissue samples were obtained as part of the surgical treatment of their tendinopathy, whereas in the remaining four, tissue samples were taken as open biopsies with no surgical treatment being performed. In 23 studies, an open biopsy was performed in isolation, in two studies microdialysis was performed in isolation, whereas in the remaining two studies, both an open biopsy and microdialysis were performed.

The control group consisted of healthy asymptomatic patients in 21 studies, cadaveric controls in three studies, patients undergoing surgery for shoulder instability in two studies, and patients with Achilles tendon rupture in one study. Nine studies used clinical pain scores to measure pain levels in individual patients; of note, only two of these studies correlated tissue findings with the pain scores.

Study Methodology and Assessing the Risk of Bias

All included studies stated clear diagnostic criteria, described their study population and control groups, and clearly described method(s) of tissue analysis. The results of the methodological quality assessment are detailed in Appendix 2. The median score was 9 (range, 7–10). Five studies produced quantitative results (four of which used microdialysis and one radioimmunoassay). Microdialysis is a minimally invasive sampling technique by which extracellular fluid is sampled and analyzed. Fourteen studies produced semiquantitative results (13 of which used immunohistochemistry and one histology), whereas eight were purely descriptive studies. Twelve of these 14 studies clearly described their method of semiquantitative analysis; two involved nerve counts and the other 10 described objective methods using more than one observer or computer analysis. Two studies did not clearly describe the semiquantitative methods and were therefore not included in the quantitative section of the results. Levels of statistical significance were stated in 16 of the 19 studies, which used quantitative or semiquantitative analysis. Of these 16,

12 studies used the 95% significance level with four studies using the 99% level. Studies that used descriptive non-quantitative methods only and/or studies that did not state the statistical significance of their results were excluded from the quantitative section of the results. Studies that did not have control groups for comparison were excluded. This methodological assessment means that the quantitative results have only included studies with minimal levels of bias.

Results

There were clear changes in the peripheral neuronal phenotype in painful human tendinopathy (Tables 2, 3). Studies have consistently shown increased overall neural ingrowth in tendinopathy versus control [6, 42, 59, 64]. Specifically there was also strong quantitative evidence showing a significant upregulation of the glutaminergic system; this relates not only to the overall tissue, but also to the nerves, blood vessels, and tenocytes (Table 3). Glutamate NMDAR1 receptors have been frequently noted in morphologically altered tenocytes in tendon tissue proper and in the peritendinous connective tissue [4, 54]. These NMDAR1 receptors have been colocalized with AChE and are frequently seen in free-nerve fibers. Tenocytes also showed a significant increase in the expression of NPY-1 and CB-1 receptors in tendinopathy. There was also good evidence that the sensory system is upregulated with substance P levels increased in tendinopathy as well as

increased numbers of substance P and CGRP (calcitonin gene-related peptide)-positive nerves. There was conflicting evidence relating to the autonomic system with one study showing a significant decrease in tyrosine hydroxylase-positive nerves and another showing no change in tyrosine hydroxylase-positive nerves.

There was relatively little direct evidence in the literature relating changes in peripheral neuronal phenotype to variations in pain symptomatology among patients; only two of the 27 studies we evaluated provided information relating to this question. One study demonstrated that substance P levels in the subacromial bursa appeared to correlate with pain levels as measured with a visual analog score. Another study demonstrated that an increased neural density in the subacromial bursa correlated with the presence of shoulder pain at rest. Although many of the other studies did not directly investigate the relationship between peripheral neuronal phenotype and pain, all remaining studies (25 of 25) compared a group with painful tendinopathy with a group of pain-free control subjects (admittedly one study's control group had asymptomatic Achilles tendinopathy that went on to rupture).

Discussion

Our knowledge of the pathogenesis of tendinopathy has advanced much in recent years, but the role of neuronal regulation in this process is still relatively poorly understood. Neuronal regulation is of key importance in tendon

Table 2. Summary of the descriptive results

Pathway	Type	Nerve	Tenocyte	Overall tissue
General		PGP 9.5 ↑↑ [6, 42]		
Sensory	Sensory	Substance P ↑ [42]	Substance P ↑ [9] NK-1R ↑ [9]	
	Opioid			
Autonomic	Sympathetic		Tyrosine hydroxylase ↑↑ [24, 68] α1 adrenoreceptors ↑ [16] Tyrosine hydroxylase and NPY → [16]	Tyrosine hydroxylase and NPY → [16]
	Parasympathetic		VACHT ↑↑ ChAT ↑↑ M2R ↑↑ [23, 25] AChE → [23] ChAT ↑ VACHT ↑ [17]	ChAT absent [68]
Excitatory	Glutaminergic			
Other	Neurotrophins		NGF ↑ BDNF ↑ p75 ↑ [13] TrkA → TrkB → [13]	BDNF ↑ [12]

PGP = protein gene product; GAP = growth-associated product; NK-1R = neurokinin-1 receptor; NPY = neuropeptide Y; VACHT = vesicular acetylcholine transporter; ChAT = choline acetyltransferase; M2R = M2 muscarinic acetylcholine receptor; AChE = acetylcholine esterase; NGF = nerve growth factor; BDNF = brain-derived neurotrophic factor; p75 = low-affinity NGF receptor; TrkA = neurotrophic tyrosine kinase receptor type 1; TrkB = neurotrophic tyrosine kinase receptor type 2.

Table 3. Summary of the quantitative results

Pathway	Type	Nerve	Blood vessel	Tenocyte	Overall tissue
General		↑neural elements [59] PGP9.5↑ [59, 64] GAP43↑ [64] ↑ neural elements with rest pain [61]			
Sensory	Sensory	Substance P ↑ [55] CGRP ↑ [59] Tyrosine hydroxylase ↓ [42] Tyrosine Hydroxylase → [55]			Substance P ↑ [34, 54] Substance P/CGRP → [58]
Autonomic	Opioid			CB1R ↑ [14]	
	Sympathetic			NPY-1R ↑ [15]	
	Parasympathetic				
Excitatory	Glutamatergic	Glutamate↑ [53] NMDAR1↑ [53]	Glutamate↑ [53] NMDAR1↑ [53]	NMDAR1 ↑ [53] Glutamate ↑ [53] VGlut2 ↑ [56] VGlut1 → [56]	Glutamate↑ ↑↑↑ [3–5, 7] NMDAR1↑↑ → [3, 54] pNMDAR1 ↑ → [54] mGluR5 ↑ [54] mGluR6 → [54]

CGRP = calcitonin gene-related peptide; CB1R = cannabinoid receptor 1; NPY-1R = neuropeptide Y receptor 1; NMDAR1 = NMDA receptor (type of glutamate receptor); VGlut1/2 = vesicular glutamate transporter 1/2; pNMDAR1 = phospho-NMDA receptor 1.

homeostasis and healing [2]. We sought to determine whether the peripheral neuronal phenotype is altered in tendinopathy; results from this systematic review suggest this to be the case. Specifically, we found strong evidence of an upregulation of the glutamatergic system in painful human tendinopathy, and we found weaker, but still suggestive, evidence that changes in the peripheral neuronal phenotype were related to variations in pain symptomatology among patients.

A significant limitation to all the research carried out on painful tendinopathy is the adequacy of the control tissue used. The gold standard control tissue would be taken from asymptomatic patients with tendinopathic tendons and this could then be compared with the tendinopathic tissue of patients with pain. Ethically this has not been possible, and the next best option is control tissue taken from healthy asymptomatic patients as was done in 21 of the 27 studies included in the review. In 11 of the 20 studies that related to the Achilles and patellar tendons, radiological assessment was used to confirm that no tendon abnormality was present in the healthy control subjects; however, in the remaining nine studies, no radiological assessment of the control subjects was undertaken, which means that some of these tendons may in fact have been tendinopathic in nature. The consequent problem is that any observed differences in the tissue between disease and control groups may or may not be related to painful tendinopathic

changes; they may be related to changes that also occur in asymptomatic tendinopathic patients.

Another limitation to the literature is that the disease subgroups studied have largely been patients undergoing surgery for the failed conservative management of their tendinopathy (23 of 27 studies); this means that the findings cannot necessarily be applied to other disease subgroups. Also, the degree to which the results of the studies were descriptive or quantitative was highly variable. As a result, only studies that used semiquantitative or quantitative methods and that stated levels of statistical significance of $p \leq 0.05$ were included in the quantitative section of the results (Table 3). Other less robust methods of result gathering were included in the descriptive results (Table 2). The variable blinding of the observers undertaking the semiquantitative tissue grading does increase the risk of study bias toward positive findings (nine of 14 studies did not state whether observers were blinded). The nature of the study results and their heterogeneity precluded a meta-analysis; the overall descriptive results (Tables 2, 3) are the reasonable limit of any synthesis in our opinion.

The cause of tendinopathy has been the subject of much heated debate over the years [40] and many different theories have been postulated [30, 47, 57, 65]. Certainly there are problems in generalizing from results relating to different tendons. The rotator cuff tendons tend to be affected increasingly in old age, whereas the patellar tendon is more

commonly affected in younger age groups. Different tendons exist in their own unique anatomical environments and are subject to different mechanical forces. This implies that there are some significant differences in the etiology of pathology in different tendons. There are, however, numerous similarities between the tendinopathies of different tendons. The histological changes are well characterized and similar across the different tendinopathies [46], whereas many of the molecular changes are also shared in common [28, 51]. Therefore, we would argue that it is valid to summarize the changes in peripheral neuronal phenotype across the different tendinopathies.

There is little doubt that the peripheral neuronal phenotype plays a key role in painful human tendinopathy; in answer to our first key question, we conclude that there is clear evidence of changes in the peripheral neuronal phenotype in painful human tendinopathy. However, the precise role that neuronal regulation plays is not yet clear. Whether changes in neuronal regulation are crucial in driving the development of symptomatic tendinopathy or whether they are more reactive secondary changes is still open to debate. The importance of nerve in growth and neuronal regulation in animal tendon healing has been well demonstrated [2]; interestingly the ingrowth of Substance P and CGRP-positive fibers was associated with increased nociception in the proliferative phase of healing, whereas the occurrence of sympathetic markers in the remodelling phase of healing was associated with decreased nociception. Work on an animal model of osteoarthritis has clearly shown the presence of ectopic neural ingrowth and this has been linked with pain generation [33]. Nerve ingrowth has also been demonstrated in degenerative spinal disease and postulated as a mechanism of pain generation [31]. Increased neural ingrowth in RCT is a consistent finding and may represent attempted tendon healing [59, 64]. The evidence of increased neural ingrowth in Achilles and patellar tendinopathy is weaker and less consistent; some studies have demonstrated increased ingrowth [6, 42] but several studies have not shown any significant differences. The reasons for these inconsistent findings are unclear; one explanation is that nerve ingrowth may diminish as a tendinopathy becomes more chronic, whereas another reasonable explanation is that the properties of the nerves and their consequent sensitivity are more important than simple nerve density.

The consistent finding of increased glutamate levels and an overall upregulation of the excitatory glutaminergic system is of particular interest. Glutamate levels have been shown to be increased in several painful musculoskeletal disorders [39, 52]. Glutamate is a key metabolite and key neurotransmitter involved in the transmission of pain

[18, 49]. Not only does glutamate have a vital role in the regulation of peripheral pain fiber sensitivity (peripheral sensitization) through its numerous receptors [18], but it also plays a key role in the sensitivity of the spinal cord and brain to pain transmission from the periphery (“central sensitization”) [63]. There is also some evidence for upregulation of the sensory system (Substance P/CGRP) but this is not as clear a finding as that of the glutaminergic system. An upregulation of the sensory system has been implicated in pain generation in both animal models and human disease [1, 31, 33, 34]. The neuropeptides Substance P and CGRP are both vasodilators and both appear to have important roles in tissue healing [19, 41]. The sensory innervation of tissue appears vital in its ability to proliferate and remodel as part of the normal healing process [37]. The histological and molecular changes in the peripheral neuronal phenotype shown in our study appear consistent with the theory that the failing healing of tendon in painful tendinopathy results in features consistent with a prolonged proliferative phase of healing. The precise reason why this apparent state of failed healing results in pain may relate to the complex dynamic of the peripheral neuronal tissue changes and the effect this has on the sensitivity of the peripheral nerve fibers.

Finally, in answer to our second question, there is a small amount of evidence that changes in the peripheral neuronal phenotype may be related to variations in pain symptomatology between patients. This is consistent with other areas of research in which changes in the peripheral neuronal phenotype have been associated with pain symptoms [50, 67]. However, we conclude that there is not enough good evidence available to answer our second key question adequately. Ultimately further research is needed to better define the role of neuronal regulation in the disease process and to better relate any changes in the peripheral neuronal phenotype to clinical pain scores.

Future studies that combine tissue analysis with clinical pain scores to study the natural history of tendinopathy and the response to different treatment modalities have the potential to help answer these important questions, including perhaps research that may lead to the identification of novel neuromodulatory drug targets. Another intriguing idea for the future is the measurement of markers of peripheral neuronal phenotype in the clinical setting with the potential of this being used as prognostic aid to guide individual patient treatment or even a surrogate outcome measure in trials.

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Appendix 1. Study details

Number	Study	Year	Journal	Pathology	Primary tissue method
1	Alfredson et al. [3]	2001	Knee Surg Sports Traumatol Arthrosc	Achilles tendinopathy	Microdialysis via open incision
2	Alfredson et al. [4]	2001	J Orthop Res	Patellar tendinopathy	Microdialysis via open incision
3	Alfredson et al. [5]	2000	Acta Orthop Scand	Humeral epicondylitis	Microdialysis via open incision
4	Alfredson et al. [6]	2003	Knee Surg Sports Traumatol Arthrosc	Achilles tendinopathy	Open biopsy
5	Alfredson et al. [7]	1999	Knee Surg Sports Traumatol Arthrosc	Achilles tendinopathy	Microdialysis via open incision
6	Andersson et al. [9]	2008	Regul Pept	Achilles tendinopathy	Open biopsy
7	Bagge et al. [13]	2009	Histol Histopathol	Achilles tendinopathy	Open biopsy
8	Bagge et al. [12]	2012	Histol Histopathol	Achilles tendinopathy	Open biopsy
9	Bjorklund et al. [14]	2011	Plos One	Achilles tendinopathy	Open biopsy
10	Bjur et al. [15]	2009	Br J Sports Med	Achilles tendinopathy	Open biopsy
11	Bjur et al. [17]	2008	Cell Tissue Res	Achilles tendinopathy	Open biopsy
12	Bjur et al. [16]	2008	Histol Histopathol	Achilles tendinopathy	Open biopsy
13	Danielson et al. [23]	2006	Microsc Res Tech	Patellar tendinopathy	Open biopsy
14	Danielson et al. [24]	2007	Microsc Res Tech	Patellar tendinopathy	Open biopsy
15	Danielson et al. [25]	2007	Life Sci	Patellar tendinopathy	Open biopsy
16	Forsgren et al. [29]	2005	Regul Pept	Achilles and patellar tendinopathy	Open biopsy
17	Gotoh et al. [34]	1998	J Orthop Res	Rotator cuff disease	Open biopsy
18	Lian et al. [42]	2006	Am J Sports Med	Patellar tendinopathy	Open biopsy
19	Schizas et al. [53]	2010	Scand J Med Sci Sports	Patellar tendinopathy	Open biopsy
20	Schizas et al. [54]	2012	J Orthop Res	Patellar tendinopathy	Open biopsy
21	Schubert et al. [55]	2005	Ann Rheum Dis	Achilles tendinopathy	Open biopsy
22	Scott et al. [56]	2008	J Orthop Res	Achilles and patellar tendinopathy	Open biopsy
23	Singaraju et al. [58]	2008	J Shoulder Elbow Surg	Long head of biceps tendon	Open biopsy
24	Xu et al. [64]	2011	Sports Med Arthrosc	Rotator cuff disease	Open biopsy
25	Zeisig et al. [68]	2009	Br J Sports Med	Humeral epicondylitis	Open biopsy
26	Tomita et al. [61]	1997	J Orthop Surg	Rotator cuff disease	Open biopsy
27	Tamai et al. [59]	2000	Clin Orthop Relat Res	Rotator cuff disease	Open biopsy

Appendix 2. Methodological quality scores for each study (q denotes question number and total denoting total score)

Author	Year	Journal	q1	q2	q3	q4	q5	q6	q7	q8	q9	q10	Total
Alfredson et al. [3]	2001	Knee Surg Sports Traumatol Arthrosc	1	1	1	1	1	1	1	1	1	1	10
Alfredson et al. [4]	2001	J Orthop Res	1	1	1	1	1	1	1	1	1	1	10
Alfredson et al. [5]	2000	Acta Orthop Scand	1	1	1	1	1	1	1	1	1	1	10
Alfredson et al. [6]	2003	Knee Surg Sports Traumatol Arthrosc	1	1	1	1	1	1	1	0	0	1	8
Alfredson et al. [7]	1999	Knee Surg Sports Traumatol Arthrosc	1	1	1	1	1	1	1	1	1	0	9
Andersson et al. [9]	2008	Regul Pept	1	1	1	1	1	1	1	1	1	1	10
Bagge et al. [13]	2009	Histol Histopathol	1	1	1	1	1	1	1	0	0	1	8
Bagge et al. [12]	2012	Histol Histopathol	1	1	1	1	1	1	1	0	0	1	8

Appendix 2. continued

Author	Year	Journal	q1	q2	q3	q4	q5	q6	q7	q8	q9	q10	Total
Bjorklund et al. [14]	2011	Plos One	1	1	1	1	1	1	1	1	1	0	9
Bjur et al. [15]	2009	Br J Sports Med	1	1	1	1	1	1	1	1	0	1	9
Bjur et al. [17]	2008	Cell Tissue Res	1	1	1	1	1	1	1	0	0	0	7
Bjur et al. [16]	2008	Histol Histopathol	1	1	1	1	1	1	1	0	0	0	7
Danielson et al. [23]	2006	Microsc Res Tech	1	1	1	1	1	1	1	0	0	1	8
Danielson et al. [24]	2007	Microsc Res Tech	1	1	1	1	1	1	1	0	0	0	7
Danielson et al. [25]	2007	Life Sci	1	1	1	1	1	1	1	0	0	1	8
Forsgren et al. [29]	2005	Regul Pept	1	1	1	1	1	1	1	0	0	1	8
Gotoh et al. [34]	1998	J Orthop Res	1	1	1	1	1	1	1	1	1	1	10
Lian et al. [42]	2006	Am J Sports Med	1	1	1	1	1	1	1	1	1	1	10
Schizas et al. [53]	2010	Scand J Med Sci Sports	1	1	1	1	1	1	0	1	1	1	9
Schizas et al. [54]	2012	J Orthop Res	1	1	1	1	1	1	1	1	1	1	10
Schubert et al. [55]	2005	Ann Rheum Dis	1	1	1	1	1	1	1	1	1	0	9
Scott et al. [56]	2008	J Orthop Res	1	1	1	1	1	1	1	1	0	1	9
Singaraju et al. [58]	2008	J Shoulder Elbow Surg	1	1	1	1	1	1	0	1	0	1	8
Xu et al. [64]	2011	Sports Med Arthrosc	1	1	1	1	1	1	1	1	0	0	8
Zeisig et al. [68]	2009	Br J Sports Med	1	1	1	1	1	1	1	0	0	1	8
Tomita et al. [61]	1997	J Orthop Surg	1	1	1	1	1	1	0	1	0	0	7
Tamai et al. [59]	2000	Clin Orthop Relat Res	1	1	1	1	1	1	1	1	0	1	9

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