

# Genetic Variation, Protein Composition and Potential Influences on Tendon Properties in Humans

B.P. Foster<sup>\*</sup>, C.I. Morse, G.L. Onambele, I.I. Ahmetov and A.G. Williams

*Institute for performance research, Manchester Metropolitan University, Crewe, UK*

**Abstract:** Sequence variations in genes that code for proteins involved in homeostatic processes within tendons may influence tendon mechanical properties. Since variants of the four genes *COL5A1*, *TNC*, *MMP3* and *GDF5* have been implicated in the aetiopathogenesis of tendinopathies, which is ultimately characterised by abnormal structural and regulatory processes, sequence variations in these four genes may also influence how the tendon functions mechanically, even in the absence of tendinopathy. For example, two reports of association between variation in the *COL5A1* gene and measures of flexibility complement reported associations between genotype and incidence of tendinopathy. Non-genetic factors such as age, body mass and physical activity status influence risk of tendon injury and physical performance potential independently from genomics, and also in gene-environment interactions. However, these non-genetic factors are often not considered in genetic association studies, probably due to their retrospective nature. Further research examining *COL5A1*, *TNC*, *MMP3* and *GDF5*, as well as other genes that may influence the maintenance of tendon homeostasis such as *COL1A1* which regulates the production of collagen type 1, the most abundant structural component of tendon is encouraged. Establishing the genetic basis of tendon properties in asymptomatic populations may advance understanding of some aspects relevant to physical performance and of the aetiology of tendinopathies. To improve understanding, accurate and reproducible assessments of tendon properties are required. However, no valid and reliable assessments of tendon properties, such as those involving *in vivo* ultrasound imaging techniques, have yet been applied to genetic association studies in humans.

**Keywords:** Genetic association studies, humans, sequence variants, tendinopathies, tendon, tendon properties.

## 1. TENDON PROPERTIES

Historically, tendons were considered bands of connective tissue with relatively no dynamic function [1-2]. More recently, numerous studies have shown that tendons provide an integral interface for transmission of forces from muscle to the bone in order to produce moments about joints, hence the mechanical properties of tendons determine the degree of joint motion in direct response to these forces [3]. From a biomechanical standpoint, external loads that act on the body are resisted by internal structures such as tendons, which undergo deformation. The degree of deformation or strain produced is related to the stress caused by these external loads and the material that it acts upon. Thus, knowledge of the mechanical properties of musculoskeletal tissues, including tendons, can assist in understanding injury risk and physical performance capabilities. The primary mechanical properties of relevance to human physical performance [4-8] include stiffness (the tendon force-displacement relationship) and the elastic modulus (the slope of the stress-strain curve in the elastic deformation region).

A biomechanical viewpoint only provides us with an analysis of forces and their 'action' or 'effects' on such structures and their function. To understand the mechanical properties of tendons, one needs to explore its structure - i.e.

both its global characteristic dimensions (e.g. cross-sectional area and length) as well as its internal structures (e.g. cross-link density) - and its dynamic function in greater detail, by examining its biochemical components. The structure or morphology of tendon, and thus its function, is controlled by cells called tenocytes that maintain the extracellular matrix (ECM) [9] and ultimately its material and mechanical properties.

The tenocytes 'sense' and respond to mechanical loads deriving from external forces. The sensitivity of this response has recently been found to be mediated by a 'mechanostat' set point, *in vitro* [9]. This preset threshold is governed by complex interactions between the cell's cytoskeleton and the ECM [9-13], ultimately giving rise to gene regulation and control over the tendon's structure and function at a molecular level. Fundamentally, the expression of functional gene products or proteins is regulated, which provides the basis for maintenance and changes in structure and function. Mechanical signalling from external loads [9, 11, 12, 14, 15] causes gene expression of various proteins involved in tendon homeostasis to vary greatly between and within animal and human populations. However, even if mechanical loading is controlled, the abundance of various proteins can still vary. This is where genetic variation is likely to influence observed/measurable differences in protein content, and thus material and mechanical properties.

Tendon pathologies or tendinopathies (including tendinosis and tendinitis) are primarily degenerative conditions that may or may not be associated with signs of inflammation [16-17]. Since research in this area is relatively

<sup>\*</sup>Address correspondence to this author at the Institute for performance research, Manchester Metropolitan University, Crewe, UK;  
Tel: 07847820666;  
E-mails: [brandon.p.foster@stu.mmu.ac.uk](mailto:brandon.p.foster@stu.mmu.ac.uk), [bpfoster1986@hotmail.co.uk](mailto:bpfoster1986@hotmail.co.uk)

extensive, reference to research studies on tendinopathies will form a small element of this review. For example, recent work has associated tendinopathies with genetic variants in proteins that serve important structural and functional roles in tendon. However, in this review, attempts will also be made to consider how those same molecular characteristics may influence tendon mechanical properties *per se*. That is to say, the same gene variants and differential gene expression of these same proteins may directly influence tendon mechanical properties either with or quite separately from their influence on incidence of injury. Indeed, there is conflicting evidence from some pathological studies regarding mechanical properties. Some studies report no significant difference in mechanical properties in patients with tendon pathology from healthy subjects [18-22], but other studies reporting significant weakening of material and altered mechanical tendon properties, with both increased stiffness [23] and decreased stiffness [24-26] observed in patients. The inconsistent results regarding human *in vivo* mechanical properties between studies on tendon pathology may be a consequence of different tendon types under investigation, (total collagen levels vary between tendon types in normal tendons [27, 28]) as well as varying subject age and sex. The latter two factors of age [29-32] and sex [33-35] are frequently reported to be highly influential on tendon material and mechanical properties.

Seven genes will be discussed in this review. The seven genes and how they relate to the five tendon proteins they produce are shown in Table 1. Four of the genes listed in Table 1 (*COL5A1*, *TNC*, *MMP3*, *GDF5*) have genetic variants reported in recent tendinopathy studies that may predispose individuals to such conditions [36-41]. In fact, all the genes that contain sequence variants shown to be associated with tendon pathology to date, encode for proteins directly involved in biological processes within tendons. Fig. (1) shows the key structural proteins found in tendon, including those associated with tendon pathologies or musculotendinous range of motion. Therefore, these genes can also be considered as candidate genes for association with fundamental tendon properties. Two studies have already reported an association between a polymorphism in one of these genes and measures of musculotendinous range of motion in humans [41, 42]. Table 2 summarises the genetic association studies that have identified a polymorphic association with tendon pathologies/musculotendinous range of motion in humans. In addition to the four

genes associated with tendinopathies (*COL5A1*, *TNC*, *MMP3*, *GDF5*), *COL5A2* will also be discussed in this review because, like *COL5A1*, it codes for a protein which is a fundamental component of the Col V molecule (quaternary protein). Type I collagen (Col I), and its two coding genes *COL1A1* and *COL1A2*, will also be discussed because it forms the major structural component of tendon, even though genetic variation has not yet been associated with tendinopathies or tendon properties. Associations have, however, been observed between genetic variation in *COL1A1* and risk of ligament injury [43, 44].

This article will review genes and proteins that are known to be related to tendon structure and/or the dynamic nature of tendon homeostasis, and the review will describe how variations within these genes may affect human tendon mechanical properties such as stiffness and elastic modulus. There is substantial evidence that tendon mechanical properties such as stiffness influence the capacity of the muscle-tendon unit to produce force during exercise of various kinds [4-8]. For example, it has been shown that a relatively stiffer muscle-tendon unit is significantly related to maximal concentric and isometric muscle performance [4]. On the other hand, during cyclical contractions low tendon stiffness (high tendon compliance) has been shown to improve muscle power and efficiency [7]. Consequently, genotype associations with tendon mechanical properties are highly likely to also influence exercise performance in a more general sense.

## 2. GENES AND PROTEINS OF INTEREST

### 2.1. Collagen Type V

#### 2.1.1. Structure of Protein and Genes

Col V is a widely distributed quantitatively minor fibrillar collagen forming between 1-3% of total collagen content of tendon ECM [45], although evidence suggests that in functional terms it is a major collagen of developing connective tissues [46]. Col V can assemble into a diverse number of molecular forms but all contain a pro  $\alpha 1(V)$  chain. This pro  $\alpha 1(V)$  chain is encoded for by the *COL5A1* gene (9q34.3) and comprises 66 exons distributed over 203.07 kilobases (kb) of genomic DNA. There is a pro  $\alpha 2(V)$  chain which is encoded for by the *COL5A2* gene (2q32.2), comprising of 54 exons and 147.98kb [47]. Together these chains form the heterotrimer protein structure of Col V ( $[\alpha 1(V)]_2\alpha 2(V)$ ), which is ubiquitous in human tendons.

**Table 1. Tendon Proteins, Genes of Focus, and Abbreviations Addressed in this Review**

Protein	Abbreviation of Protein Used in this Review	Genes of Focus in this Review	Abbreviation of Gene of Focus
Type V collagen	Col V	collagen, type V, alpha 1 collagen, type V, alpha 2	<i>COL5A1</i> <i>COL5A2</i>
Tenascin C	Ten C	tenascin C	<i>TNC</i>
Matrix metalloproteinase-3	MMP-3	matrix metalloproteinase 3 (stromelysin 1, progelatinase)	<i>MMP3</i>
Growth/differentiation factor 5	GDF-5	growth differentiation factor 5	<i>GDF5</i>
Type I collagen	Col I	collagen, type I, alpha 1 collagen, type I, alpha 2	<i>COL1A1</i> <i>COL1A2</i>

Note: the two collagen proteins comprise numerous protein chains and therefore are dependent on more than one gene.

**Table 2. Summary of Genetic Association Studies that have Identified a Polymorphic Association with Tendon Pathologies/Musculotendinous Range of Motion in Humans**

Gene	Participants	Gene Variant	Phenotype	Findings/Observations	Study
COL5A1	White Caucasian. 72 with chronic ATP, 39 with acute Achilles tendon rupture. 129 control.		Chronic ATP.	Individuals with A2 (C) allele gene variant of this gene are less likely of developing symptoms of chronic Achilles tendinopathies	Mokone <i>et al.</i> , [39]
	White Caucasian. 85 Australian and 93 South African patients with ATP, respectively. 210 Australian and 132 South African control subjects.			Individuals possessing 'CC' genotype had decreased risk of developing chronic ATP compared with those individuals with T allele (TC or TT genotypes) in both populations	September <i>et al.</i> , [38]
	White Caucasian. 50 with chronic ATP, 35 with acute Achilles tendon rupture. 34 control.	Bst UI RFLP within 3' untranslated region (UTR) (rs12722 C/T)	Standing leg raise, sit- and-reach.	Individuals with CT genotype were found to be less flexible than homozygous individuals	Collins <i>et al.</i> , [41]
	White Caucasian. 325 healthy and physically active cohort.		Sit-and- reach.	Older individuals ( $\geq 35$ years) homozygous for the C allele showed greater flexibility	Brown <i>et al.</i> , [42]
TNC	White Caucasian. 72 with chronic ATP, 42 with acute Achilles tendon rupture. 127 control	GT dinucleotide repeat polymorphism within intron 17	Chronic ATP. Acute Achilles tendon rupture.	Individuals with 12 and 14 GT repeats appear to have 6-fold risk of developing Achilles tendon injuries. 13 and 17 repeats were underrepresented	Mokone <i>et al.</i> , [37]
MMP3	White Caucasian. 75 with chronic ATP, 39 with acute Achilles tendon rupture.	(rs679620) A/G transition at nucleotide position 28 within exon 2, (rs591058) T/C transition at nucleotide position 1547 within intron 4, (rs650108) G/A transition at nucleotide position 495 within intron 8.	Chronic ATP.	GG of rs679620, CC of rs591058, AA of rs650108 genotypes overrepresented in individuals with ATP but no association found independently with individuals with acute Achilles tendon rupture. Additional observation- inferred haplotype GC greater frequency in ATP, haplotype ATG greater in control subjects	Raleigh <i>et al.</i> , [40]
GDF5	White Caucasian. 171 recruited. Australian population- 59 with chronic ATP. South African population-73 with chronic ATP, 39 with acute Achilles tendon rupture. Australian population- 142 control, South African population-96 control.	(rs143383) T/C substitution of functional promoter in 5' UTR	Chronic ATP. Acute Achilles tendon rupture.	Individuals with TT genotype have twice the risk of developing both chronic ATP and acute Achilles tendon rupture	Posthumus <i>et al.</i> , [36]

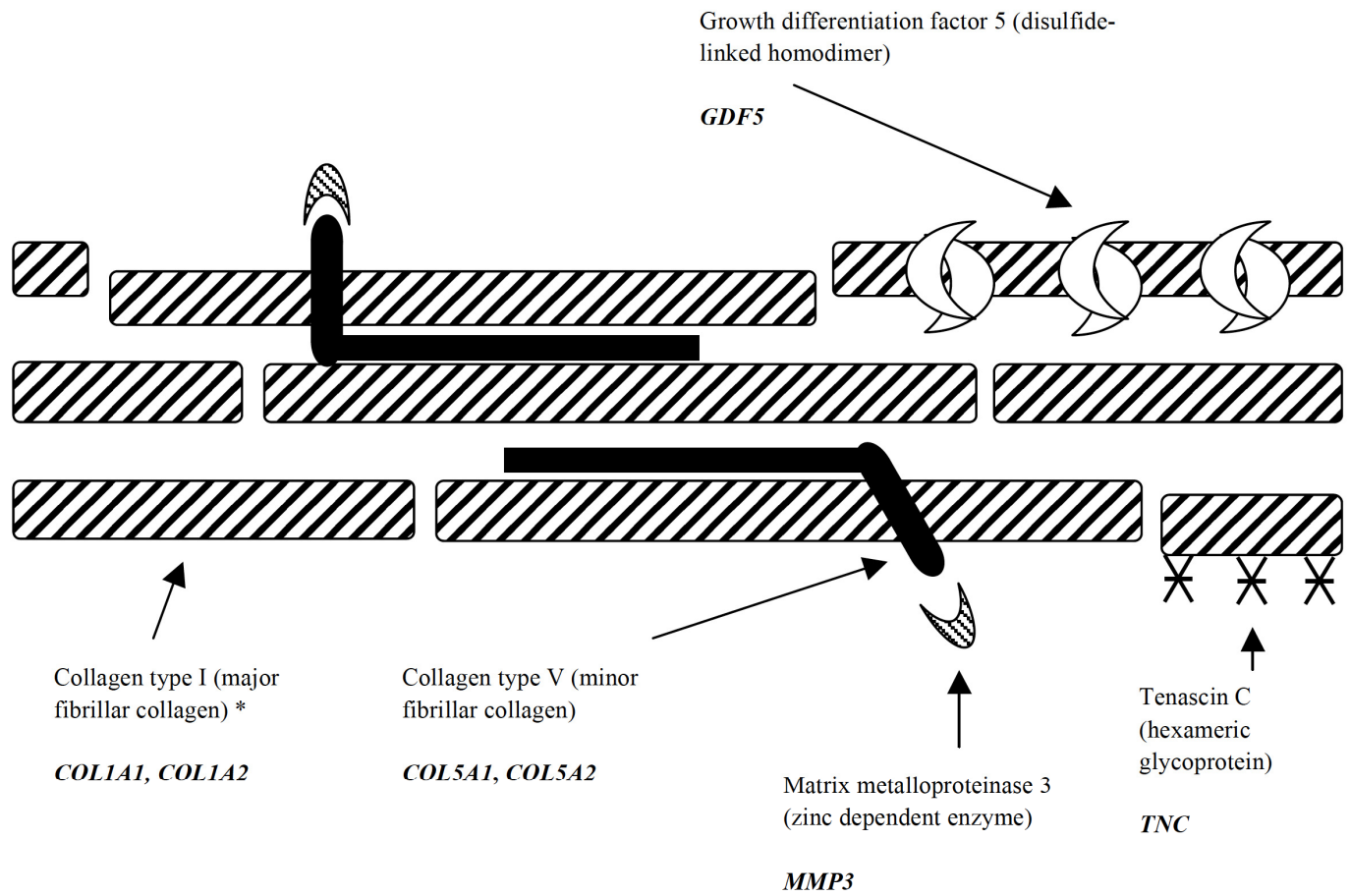
\*ATP = Achilles Tendon Pathology.

### 2.1.2. Function

Col V plays a functionally important role in tendon *via* its relationship with Col I fibrils, in that it is thought to co-polymerise with Col I fibrils to form heterotypic fibres, and thereby organises and regulates the diameter of these fibres [48-51] as well as forming intermolecular cross-links with Col I fibrils [52]. Interaction of Col V with Col I in *in vitro* self-assembly assays has shown a decrease in the diameter of

fibrils with increasing amounts of Col V [53], possibly due to an increase in nucleation sites in the thin filaments of Col V for a given quantity of Col I [49]. These sites serve as steric hindrances for the addition of Col I molecules, through amino terminal domains which project out, thereby regulating lateral growth and hence diameter [51, 52].

Both fibril diameter [29, 54, 55] and extent of cross-linking [56-59] are positively correlated with mechanical



**Fig. (1).** Major microstructural components of tendons associated with tendon pathologies/musculotendinous range of motion, identifying related genes. \* No genetic association with tendon pathologies/tendon properties yet reported, but its presence in this figure is warranted because it is the major structural component of tendon. Adapted from Collins and Raleigh (2009).

properties of tendon such as stiffness and Young's Modulus, in animal models. Additionally, this correlation has been reported *in vivo* in relation to fibril diameter in humans [55]. There appears to be an optimum level of permanent cross-linking, above which there is a decrease in mechanical strength [53, 60, 61]. However, these studies investigated age-related declines in mechanical properties and the same relationship with cross-linking may not exist in a cross-section of a younger population. Reduced Col V content has been reported to compromise the diameter of the collagen fibril in *in vitro* cultures [62, 63] and consequently may reduce the material properties of tendon such as maximum stress and linear modulus [53].

### 2.1.3. Evidence of Polymorphic Associations with Tendon Pathologies

The first study to report an association between variation in the *COL5A1* gene and tendon pathology [39] identified the *COL5A1* gene as an ideal candidate genetic marker of Achilles tendinopathies because it is on the same locus of genomic DNA as the ABO gene, which has been reported to be associated with tendon injuries in Hungarian and Finnish patients [64, 65]. Two restriction fragment length polymorphisms (RFLPs) were identified within the 3' untranslated region (UTR) of the *COL5A1* gene (*Bst*UI and *Dpn*II) that had no known role in the expression or function of Col V. An association was found between the *Bst*UI

RFLP (rs12722) and Achilles tendon pathology (ATP), and more specifically chronic tendinopathy without rupture (the C allele was protective against ATP). As the authors rightly stated, this association does not show conclusively that Col V is involved in the development of these pathologies, and it is of course likely that there are numerous genetic variants that contribute to the overall heritability of such conditions [66]. It is also possible that non-genetic factors influenced the results of the study described in this paragraph [39], as body mass and physical activity were not controlled participant selection criteria.

A subsequent study investigated the same variant of the *COL5A1* gene but in two separate white populations in South Africa and Australia [38] and the results generally concurred with the initial association study [39], in that rs12722 (*Bst*UI RFLP) within the 3' UTR was associated with Achilles tendinopathies and associated with individuals who possess a 'T' allele at this locus. Thus, individuals who were homozygous for the 'C' allele were apparently less likely to develop the condition. The authors intelligently attempted to investigate a combination of additional markers or neighbouring alleles in the same sequence region of the 3' UTR of the *COL5A1* gene, known as an 'inferred' haplotype, in order to provide more information as to the predisposing causative factor. The haplotype consisting of markers rs12722 and rs3196378 (alleles 'T' and 'C' respectively) was

significantly overrepresented in the South African tendinopathy group but not in the Australian group. The DNA sequence that contains the 'C' allele at the rs3196378 marker forms part of a miRNA recognition sequence. miRNA are key regulators of gene expression at a posttranscriptional level by inhibiting translation or inducing mRNA cleavage [67, 68]. Consequently, protein expression may be modified and in this instance one could speculate that *COL5A1* expression may be altered, leading to suboptimal levels of Col V protein and ultimately a compromised collagen fibre and healing process. Regarding *COL5A2*, no polymorphisms as of yet have been associated with tendon pathologies or function.

### 2.1.4. Possible Influences on Tendon Properties

Two recent studies considered a measurable *in vivo* phenotype that may link the *COL5A1* gene variant to tendon injuries, by investigating flexibility - a possible immediate phenotype [41, 42]. Flexibility is an established determining factor for patellar tendinopathies in active populations [69, 70]. Genetics has been reported to contribute substantially to the variability of certain flexibility phenotypes [71, 72], and although that is not the case in other studies [73, 74], associations between gene variants and flexibility are therefore plausible. *COL5A1* was hypothesised to be associated with flexibility following reports that mutations in the *COL5A1* gene have been implicated in Ehlers Danlos syndrome, a condition characterised by joint hypermobility [75]. These disease-associated rare mutations may produce non-functional *COL5A1* and *COL5A2* alleles leading to haploinsufficiency of *COL5A1* and *COL5A2* mRNA, predictably resulting in the synthesis of around half the amount of normal Col V protein [75-78]. Phenotypically, this may result in abnormally large collagen fibrils [79] and impaired mechanical properties of tendon. In the first study, an association was reported between the common polymorphism rs12722 and flexibility [41]. Individuals heterozygous (CT) for this genotype were less flexible than homozygous individuals of either allele, however the study sample contained significant heterogeneity in terms of tendon injury history - i.e. participants with tendinopathies, history of rupture, and no history of tendon injury were combined in the genotyping results. Furthermore, measures used to quantify flexibility, such as an instrumental standing leg raise [80] and a trunk flexion sit and reach test [81], are rather crude measures of the function of the muscle-tendon unit as a whole, and certainly do not provide precise data on the mechanical properties of the tendon *per se*. Regarding the instrumental standing leg raise test, subjectivity [82] and specifically the perception of pain onset, as well as abnormal defence reactions [83], could be particularly relevant to the participants with history of Achilles problems. However, the main critical comment on the instrumental standard leg raise test is that the measured phenotype is a composite of various factors including muscle and tendon stiffness and does not take into account the dimensions of the structures so the mechanical properties cannot be determined. Additionally, any kind of sit-and-reach test is a composite measure of various factors contributing to 'flexibility', including muscle-tendon unit stiffness [84], limb lengths and proportions [85]. Thus, we conclude that while their approach was a useful step in the study of the genetics of

flexibility and range of motion, the mechanics of the tendon *per se* were clearly not determined by Collins *et al.*, [41] so potential associations between tendon properties and genes coding for proteins expressed in tendon could not be investigated directly in that study. The previous critique is accentuated by a report showing the mechanical properties of the series elastic component (tendon-aponeurosis) are independent of the parallel elastic component (passive muscle stiffness) *in vivo* [86].

In the second study, Brown *et al.*, [42] investigated the *COL5A1* rs12722 polymorphism and sit-and-reach performance in a healthy and physically active cohort (325 Caucasian subjects). Individuals homozygous for the 'C' allele had greater flexibility, but this was only observed in the older ( $\geq 35$  years) subjects where sex and *COL5A1* genotype accounted for approximately 23% of the variance. As per the previous study, some factors which may affect the material and mechanical properties of the tendon were not considered. For example, circulating oestrogen was not assessed in the female subjects - chronic oestrogen levels can influence tendon stiffness [87]. Also, a lack of detailed information regarding the habitual physical activity levels of the older subjects was another limitation noted by Brown *et al.*, [42] themselves, because tendon stiffness can increase [15, 88] with higher physical activity and decrease [89, 90] with lower activity.

In conclusion for Col V, it is indeed possible that variations within the genes that encode for the molecular components of Col V may influence a tendon's material and mechanical properties, although such gene variants have not yet been shown to influence tendon properties *per se*. Since Col V expression levels appear critical in determining a tendon's fibre structure through diameter and cross-linking [52, 63], several testable hypotheses regarding genetic variants and mechanical properties of tendon such as stiffness, maximal strain and elastic modulus are likely to be tested in the coming years.

## 2.2. Tenascin-C

### 2.2.1. Structure of Protein and Gene

Ten C is an ECM glycoprotein consisting of 6 monomers, expressed in tissues bearing high tensile stress, such as tendons and myotendinous and osteotendinous junctions [91] and as with the collagen fibre, is an important structural component of tendon [92, 93]. This protein is known to be sensitive to mechanical loading *in vitro* [94]. It is encoded by the *TNC* gene (9q33.1), which comprises 28 exons spanning 97.63 kb of genomic DNA [47].

### 2.2.2. Function

Ten C is a structural component of tendons, yet is an elastic protein [95], and as it is expressed in mechanically loaded tendons it may contribute to increased elasticity of the ECM [92, 96, 97] *via* increased gene expression in response to stretch [98]. In addition to its structural roles, Ten C performs various regulatory roles within the ECM. Due to its modular structure, the protein is able to interact with various other proteins involved in ECM homeostasis, as well as playing an important role in regulating cell-matrix interactions [99]. It is also believed that Ten C plays an invaluable role in regulating proper alignment and

organisation of the collagen fibres *in vitro* [95]. Therefore, when Ten C is expressed it may contribute to an increased crimp angle - a region-specific morphological feature of collagen fibres associated with the mechanical properties of tendon.

Ten C may also be a strong candidate for involvement in the aetiology of musculoskeletal injuries, as expression of the *TNC* gene has shown to be altered with human tendinopathies, determined by immunoblotting [100, 101]. This may be particularly relevant when engaging in intensive activity following inactivity. Inactivity decreases tendon stiffness *in vivo*, particularly at the muscular end of human tendon [89]. Yet Ten C expression relies on mechanical loading and as it is an 'elastic' protein, a relative decrease in stiffness due to inactivity or insufficient mechanical loading makes intuitive sense if Ten C is more highly expressed. Indeed, the myotendinous interface has been reported to be mechanically the most vulnerable site for injury [102, 103]. Thus, during reloading after inactivity the overall extensibility (strain to failure) would decrease increasing the risk of tendon rupture, due to experiencing greater strains for a given load [104].

### 2.2.3. Evidence of Polymorphic Associations with Tendon Pathologies

One study investigated a guanine-thymine (GT) dinucleotide repeat polymorphism for potential association with the risk of incurring both chronic Achilles tendinopathies and Achilles tendon rupture [37]. This polymorphism is a tandem repeat, consisting of a 2 base pair sequence repeated a varying number of times within a non-coding region (intron 17). Variants containing 12 and 14 GT repeats were overrepresented in subjects with tendinopathies, while variants containing 13 and 17 repeats were underrepresented. The control group had been active in high impact sports for a considerable time (11.5 years) and were currently engaging in ~5 hours per week, and so their apparent resistance to tendinopathies was unlikely to be due to a significantly lower exposure to high impact loading. Thus, a genetic influence may indeed exist, although replication of these data would be a valuable development. Furthermore, whether the *TNC* polymorphism is involved in causative mechanisms is still debatable.

### 2.2.4. Possible Influences on Tendon Properties

Even though the GT repeat polymorphism in intron 17 is not part of the coding sequence, intronic variations may influence the binding of proteins involved in gene transcription, thus affecting gene expression. As Ten C expression has been reported to be up-regulated in certain pathological conditions [105-107], it could be postulated that the 12 and 14 GT repeats within intron 17 of the *TNC* gene may overexpress Ten C, increasing the elastic properties of the myotendinous unit, as well as reducing the ultimate tendon strain to failure for a given load [97]. Thus, *TNC* is a candidate gene with regards to determining the degree of passive stiffness/compliance of tendon.

## 2.3. Matrix Metalloproteinase 3

### 2.3.1. Structure of Protein and Gene

MMP-3 (otherwise known as stromelysin-1) is part of a group of 5 domain structures of zinc-dependent enzymes

known as Matrix Metalloproteinases (MMPs), characterised according to the type of zinc binding. Structurally, the MMP-3 protein constitutes a multi domain structure made up of a propeptide, a catalytic N-terminal domain and a haemopexin-like C-terminal, all of which combine to form functional MMP-3 which has the capacity to interact with its substrates [108]. The MMP-3 protein is encoded for by the *MMP3* gene (11q22.2) which is 10 exons in length and covers 7.79kb [47].

### 2.3.2. Function

MMP-3 plays a crucial role in the normal development, repair and remodelling of connective tissues, and ultimately plays regulatory roles in maintaining ECM homeostasis, through proteolytic activity. This is achieved by catalysing the degradation of both ECM and non-ECM proteins [109-111]. MMP-3 hydrolyses multiple substrates including different types of intact fibrillar collagens, proteoglycans and a wide range of ECM components [112]. The ECM is in a state of dynamic equilibrium between synthesis and degradation [113], and its gene expression has shown to be increased by mechanical loading *in vitro* [114, 115]. Recently, ECM regulation has been shown to be determined by a combination of duration and magnitude of the mechanical stimulus *in vitro* [13], and in an *in vivo* rodent model [116], which potentially represents the impact of differing forms of voluntary exercise on tissue remodelling processes in humans.

The expression level of MMP-3, which may be regulated at the transcriptional, translational, or posttranslational levels by interaction with inhibitors [117], appears to differ between normal and highly stressed tendons, as well as tendons displaying pathological characteristics, determined by histological analyses. In highly stressed tendons, expression levels are elevated compared to normal tendons in animal models [13, 118, 119], which is thought to represent a repair or maintenance function that may be associated with an underlying degenerative process [117]. In addition, 'stress-shielding' or load deprived tendon has shown to increase the expression of MMP-3 mRNA in relation to normal cadaveric tendon tissue samples, determined *in vitro* [118, 120, 121]. Collectively, these studies point toward a 'U' shape relationship between load and MMP-3 expression. The extremes of this relationship may cause a loss of mechanical function, which may be related to the subtle degradation of ECM components, notably those involved in cross-linking and/or stabilisation of the tendon structure [121], such as minor collagens including Col V as well as proteoglycans.

In contrast, a lower level of MMP-3 expression compared to normal tissue samples has been reported in human tendon displaying pathological characteristics [100, 117, 122-124]. These observations may represent a failure of the normal matrix remodelling process [125]. It should be noted that even in normal human tendon, there is a significant difference between sexes where males have twice the amount of resting mRNA expression levels of MMP-3 compared to females [126], which may indicate an impaired ECM maintenance and a weakening of the material properties in females, leading to increased injury susceptibility [127].

Even though the evidence is generally consistent across studies, with regards to the MMP-3 gene expression in different mechanical environments and in pathologies, there are some complexities. For instance, increased MMP-3 mRNA expression does not mean that a given amount of MMP-3 protein will be produced due to post-transcriptional and post-translational regulation [112, 125].

### **2.3.3. Evidence of Polymorphic Associations with Tendon Pathologies**

Gene variants have been investigated in the *MMP3* gene which have the potential to substantially alter its expression [128], particularly the 5A/6A polymorphism within the promoter region of human *MMP3*. This polymorphism has been associated with a number of pathological states [129-131]. The association between gene variants in *MMP3* and tendon pathology was first postulated when immunochemically detectable MMP-3 protein was lower in a 'normal' region of Achilles tendon tissue in patients with a degenerate core region nearby, compared to normal control tissue [100]. This suggests these patients with tendinosis were predisposed to developing the condition due to inherently reduced MMP-3 protein levels.

One study to date has reported an association between variation in the *MMP3* gene and Achilles tendinopathy [40]. Three SNP's spanning most of the gene were identified as being potentially informative, as they are part of all four major haplotypes within the *MMP3* gene (one exon SNP - rs679620, two intron SNP's - rs591058, rs650108). All three *MMP3* variants were found to be associated with Achilles tendinopathy individually, and as inferred haplotypes - particularly between the rs679620 and rs591058 gene variants. These two variants were found to be in almost perfect linkage disequilibrium. In contrast, the 'ATG' inferred haplotype containing all three SNP's were significantly underrepresented in the tendinopathy group compared to the control group, suggesting this combination has a protective effect against the development of Achilles tendinopathy.

Raleigh *et al.*, [40] were the first to demonstrate an interaction between variants on two different genes, vis-à-vis the development of Achilles tendinopathy (all three SNP's of the *MMP3* gene and the marker rs12722 of the 3' UTR region of *COL5A1* gene). The rs679620 marker of the *MMP3* gene and the rs12722 marker of the 3' UTR region of *COL5A1* gene represent the best pair of genotypes for estimating the risk for Achilles tendinopathy, with the 'G+T' allele combination associated with tendinopathies. However, the authors do not address how the *MMP3* variants alone, or as haplotypes and inferred haplotypes between different genes, cause an increased/decreased risk of tendinopathies. Nevertheless, they do suggest that the rs679620 variant of *MMP3*, which is a non-synonymous polymorphism, may influence the downstream function of the mature MMP-3 enzyme and its activation [129] due to the subtle change in the amino acid coding and its interaction with other amino acids ('G' allele=glutamate, 'A' allele=lysine). The 'G' allele may encourage elevated levels of MMP-3 expression via increased MMP-3 activation, as a result of altered interaction with other amino acids in the propeptide region.

### **2.3.4. Possible Influences on Tendon Properties**

As the *COL5A1* BstUI RFLP was shown to be associated with human flexibility, the *MMP3* rs679620 variant was investigated for this same association, though no association was evident [132]. The precise rationale for investigating a link between this gene variant and flexibility is unclear, although as a link was previously identified between the *MMP3* gene variant and Achilles tendon injuries [40] and as flexibility has been reported to be a possible risk factor for these injuries [133], the investigation seems justified. It must be noted that the flexibility phenotype assessed was a measure of musculoskeletal passive flexibility, which encompasses tendons, ligaments, joint capsules, aponeuroses and fascia sheaths, as well as the muscle and not necessarily just the tendon. Thus, as previously mentioned in section 2.1.4 there are limitations to the techniques used for measuring flexibility in these studies.

As the mechanical properties of tendons are primarily a function of the ECM, and because a majority of ECM components are substrates for the proteolytic activities of MMP-3 [109], it may be that MMP-3 expression would contribute to the material integrity, and thus tendon mechanical properties. It may be that elevated expression levels of the *MMP3* gene indicate a degenerative environment, putting the ECM in a state of imbalance with a greater rate of degradation compared to synthesis. The tendon homeostatic abilities would thus be compromised and substrates involved in cross-linking and stabilisation of the collagen fibril (Col V) may be degraded, ultimately weakening the material properties and resulting in a reduction in matrix stiffness [56-57].

## **2.4. Growth Differentiation Factor-5**

### **2.4.1. Structure of Protein and Gene**

GDF-5 is a member of the transforming growth factor (TGF) super-family, encoded for by the *GDF5* gene (20q11.22) of which its entire coding region comprises 4 exons and is approximately 21.42kb in length [47]. Structurally, it is a 'dimer' consisting of two monomers interlinked by disulfide bonds. Mature forms of the protein are approximately 110-140 amino acids in length and seven cysteine amino acid residues are involved in creating its rigid structure [134].

### **2.4.2. Function**

GDF-5 is involved in maintenance, growth and repair of bones, cartilage and musculoskeletal soft tissues including tendon [135-137]. When GDF-5 was first investigated for its possible role in tendon biology, it was found to possess a unique ability to induce a tendon-like tissue rather than cartilage and bone, when implanted intra-muscularly in rats [138]. Further investigations found a significant role of GDF-5 in tendon within rodent models with induced Achilles tendon injuries. Firstly, GDF-5 was found to enhance tendon healing and tensile strength of the tendon when implanted on collagen sponges, in a dose-dependent manner [139]. Further studies examined the ultrastructural, compositional and mechanical characteristics of the Achilles tendon in rodents deficient in GDF-5, and found the maximum load to failure decreased possibly due to



significantly less collagen, an increase in irregularly shaped Col I fibrils and compromised material behaviour (decrease in strength and stiffness) [140-142]. Therefore unsurprisingly, GDF-5 has been shown to increase mechanical strength in these rodent models [143-146]. These studies are further supported at a cellular and gene level by studies showing an improved collagen organisation with GDF-5 treatment [147, 148], as well as an increased expression of genes and synthesis of the components of tendon ECM, in particular, Col I, Ten C and MMP-3 [149].

#### 2.4.3. Evidence of Polymorphic Associations with Tendon Pathologies

The human *GDF5* gene contains mutations known to cause a number of rare inherited disorders, including acromesomelic chondrodysplasia of the Hunter-Thompson and Grebe types as well as Du Pan Syndrome, all of which are characterised by musculoskeletal abnormalities, including shortened limb bones, brachydactyly and severe joint dislocations [150-152]. The hypothesised involvement of *GDF5* in tendon pathologies derives from this evidence, as it was postulated that the observed joint dislocations may be attributed to abnormalities in tendons [140]. As well as genetic mutations within the *GDF5* gene, a functional promoter SNP (rs143383; T/C) of the 5' UTR of the *GDF5* gene has been associated with multifactorial disorders, such as osteoarthritis at different joint locations across different ethnic groups [153-156], congenital dislocation of the hip [157], as well as total body height, hip axis length and fracture risk [158, 159], and lumbar disc degeneration [160]. In articular cartilage of individuals with osteoarthritis, there was a 12% lower expression of GDF-5 associated with the 'T' allele at this SNP marker compared to the 'C' allele [161]. So, a reduction in the expression of the *GDF5* gene associated with the 'T' allele may contribute to tendon pathologies, and this has been investigated by one study.

In a case-control study, an association was reported between the *GDF5* SNP rs143383 referred to above and the risk of ATP [36]. Individuals of 'TT' genotype were found to have approximately twice the risk of developing ATP within an Australian population independently and when combined with a South African population, which probably means it is less likely to be a false positive observation. No significant association between genotype and higher risk was shown in the South African cohort alone, although the observed odds ratio was still similar (~1.7). The relatively small sample size of the Australian tendinopathy group (n=59) as well as the different physical characteristics (body mass and BMI) between the ATP and control groups of both populations, are perhaps limitations of the study. However, the odds ratios and confidence intervals observed suggest a robust association and these findings complement those studies demonstrating the impact on gene expression of the *GDF5* variant in question [161-163].

#### 2.4.4. Possible Influences on Tendon Properties

It may be hypothesised that the material properties of the tendon are compromised in the presence of the rs143383 'T' allele variant, i.e. a reduction in tensile strength and stiffness. GDF-5 may be involved in collagen cross-linking by promoting the proteolytic activation of lysyl oxidase [164] as well as mediating the collagen structure and organisation, by

increasing the thickness of collagen fibrils [140]. Collagen cross-linking and diameter per se, have been shown to increase tendon matrix stiffness in animal models [54, 56, 57] and humans [55], so the 'T' allele variant of *GDF5* may hinder these processes and thus reduce tendon stiffness. Therefore, if gene variants within the *GDF5* gene influence tendon material and mechanical properties, it is likely to be *via* mediating the growth of other structures, in particular Col I fibrils.

## 2.5. Collagen Type 1

### 2.5.1. Structure of Protein and Genes

Collagen is the main protein constituent of tendon tissue and is reported to make up 65-75% of a tendon's dry mass [165] in cadaver tissue and more recently ~90% of *in vivo* patella tendon biopsies in humans [166]. Collagen comprises fibrillar collagen molecules containing more than 95% Col I [27]. This collagen protein is encoded for by the *COL1A1* gene (17q21.33), which constitutes 52 exons and is 18.34kb in length, and to a lesser extent by the *COL1A2* gene (7q21.3), which constitutes 52 exons also, and is 36.67kb in length [47]. The *COL1A1* gene encodes for the alpha ( $\alpha$ ) 1 chain, while the *COL1A2* gene encodes for the  $\alpha$  2 chain. Two  $\alpha$  1 chains and one  $\alpha$  2 chain combine to form a heterotrimer protein structure.

### 2.5.2. Function

Col I is a major protein constituent contributing significantly to the structural integrity of soft tissues such as cruciate ligaments, joint capsules and tendons, *via* the formation of strong parallel bundles of fibres. Col I fibrils and fibres are well recognised to be involved in tensile strength and the stiffness of tendon matrix, based on its intra- and inter- molecular cross links, orientation, density, diameter and length, all of which have been shown to affect the mechanical properties of the tendon as a whole in animal models [54-59, 167, 168].

### 2.5.3. Possible Polymorphic Associations with Tendon Pathologies

Mutations as well as single nucleotide polymorphisms in the *COL1A1* gene, particularly a SNP affecting the Sp1 binding site in the first intron of the *COL1A1* gene (+1245; G/T; rs1800012), have been associated with lower bone mineral density and osteoarthritis [169-176] as well as being implicated in the disease *osteogenesis imperfecta (OI)* which is characterised by fragile collagen structures [177-179]. Additionally, a point mutation in the *COL1A2* gene (nucleotide position 1121) that substitutes serine or cysteine for glycine residues (C-to-T transition and G-to-T transversion, respectively), also leads to the OI phenotype [180]. Consequently, sequence variants such as these, and others that may have a less clinically evident but still important influence, might be associated with soft tissue injuries. Indeed, associations have been reported between SNP's in the *COL1A1* gene at the intronic Sp1 transcription factor binding site and the risk of cruciate ligament ruptures and shoulder dislocations [43, 44], as well as upper limb muscle strength in elderly men [175]. These associations may be mediated through reduced Col I content or a weaker form of Col I, but as of yet no genetic association has been made with tendon pathologies or tendon properties.



### 2.5.4. Possible Influences on Tendon Properties

No association has yet been reported between variation in the *COL1A1* gene and tendon pathologies or properties. It is known that a SNP within the intronic Sp1 binding site (rs1800012) increases transcriptional activity of the *COL1A1* gene, resulting in abnormal ratios of the  $\alpha 1(1)$  protein relative to  $\alpha 2(1)$ , which possibly gives rise to weaker homotrimers being formed (three  $\alpha 1(1)$  chains) instead of the conventional heterotrimers (two  $\alpha 1(1)$  and one  $\alpha 2(1)$  chains) [178]. It has also been reported that two polymorphisms in the proximal promoter of *COL1A1* are in linkage disequilibrium with the Sp1 polymorphism [181], and in fact form an extended haplotype with the Sp1 polymorphism to regulate *COL1A1* transcription. This is achieved by affecting the binding affinity of important regulating factors, such as Sp1, with the 'T' allele at the Sp1 binding site found to have a higher DNA binding affinity than the 'G' allele [171]. Consequently, individuals who carry a 'T' allele instead of a 'G' at this SNP, highly express *COL1A1* and thus possess a greater proportion of the weaker  $\alpha 1$  homotrimers, may be more likely to have a compromised tendon internal structure. It has also been suggested that overproduction of Col 1  $\alpha 1$  chains in tendon might result in a higher tensile strength [44], although that statement contradicts the mechanism just outlined and is not expanded upon by the authors.

It is unlikely that tendon properties are affected solely by the Sp1 polymorphism in intron 1 of the *COL1A1* gene. It is more likely that the extended haplotype influences the transcription of *COL1A1* [171] and the material quality of the Col I fibril, with individuals carrying a 'T' allele at the Sp1 polymorphism ultimately producing higher gene activity, which might contribute to a more adversely affected Col I fibril. In addition, other genes such as those already reviewed need to be considered at the same time.

### CONCLUSION

Referring to the studies examining gene variants and their associations with tendinopathies, it appears there is no single causative gene variant that predisposes individuals to tendinopathies. This suggests tendinopathies are likely to be polygenic. This concurs with the expectation that a multitude of genes, their associated proteins, and their heterogeneous interactions are required to maintain normal tendon structure and homeostasis through development, regeneration and normal function [62, 168]. Therefore, it is likely, even after controlling for other parameters such as gender, age and habitual physical activity, that the intrinsic material (structural and regulatory) and mechanical properties of a tendon, quite apart from tendinopathies, are similarly influenced by polygenics.

Caution must be taken when interpreting the findings of these genetic association studies on tendinopathies. Investigations into the genetic factors involved in their aetiology, thus far, are very much in their infancy [182]. To date, all studies examining the genetic factors involved in tendinopathies investigate the pathology of the Achilles tendon, so whether these findings are applicable to other types of tendon is unknown. Also, the studies fail to control for all pertinent environmental factors, which may influence inter-individual variability in risk of tendinopathy. Non-genetic factors such as age, body mass and physical activity

of participants, all of which may affect the risk of incurring tendon injuries (independent of genetic predisposition), are usually not controlled for when recruiting individuals for these studies. This is mainly because the studies are retrospective in nature, rather than prospective, in that the pathological condition was evident before genetic variables were considered. Lastly, these genotype-phenotype associations have only been reported in people of one form of geographic ancestry (white Caucasian), and it remains to be investigated whether these associations will be observed in peoples of other geographic ancestry. Additionally, to improve the strength of these relationships intra-ancestrally, it would be beneficial to conduct twin/family studies to provide estimated inter-individual variability that is inherited, which has not yet been done [182].

Having further highlighted the role of ECM proteins and their possible link to tendinopathies, it appears they do not act as one single entity but subtly interact to form interlinked structures (Fig. 1) and govern dynamic processes within the ECM. Col V fibrils combine with Col I fibrils to regulate the diameter of the fibres [49] with Ten C playing an invaluable role in regulating the proper alignment and organisation of the collagen fibres [95]. MMP-3 may degrade minor collagens such as Col V, which may alter the cross-linking and stabilisation of the tendon structure [112]. And lastly, an improved collagen organisation and cross-linking density was observed with the addition of GDF-5 [147, 164]. The common theme in these associations is the integrity of the collagen fibre in conjunction with organisation, diameter and cross-linking, all of which have been linked to tendon properties such as tendon matrix stiffness [54, 183].

It is therefore reasonable to suggest that variants in genomic DNA sequence within these and other relevant proteins are likely to contribute to observed phenotypic variations in the tendon, most notably the mechanical properties, which may have implications for physical performance capabilities and the risk of incurring musculoskeletal injuries. However, no study has yet attempted to investigate genetic influences upon tendon properties *per se* in an asymptomatic population.

### FUTURE

When investigating a gene variant's influences on tendon properties, it is important to negate factors other than genetics that are likely to contribute to tendon phenotypes. To establish a valid and reliable association between a gene variant and tendon mechanical properties, experimental error must be minimised and appropriate phenotype measurements utilised, which has not been adequately achieved in the previous genetic association studies investigating flexibility [41, 42]. It would be more appropriate to measure the overall stiffness of the muscle-tendon unit to assess flexibility using, for example, passive isokinetic dorsiflexion adopted by Morse *et al.*, [184] to assess the human gastrocnemius muscle-tendon unit. This comprehensive *in vivo* assessment utilises techniques such as dynamometry, electrogoniometry, electromyography (EMG) and ultrasonography. The mechanical properties of the tendon itself can be assessed *in vivo*, which would be in line with the objectives of associating genetics with tendon properties. A thorough and highly reliable assessment is detailed by Pearson and

Onambele [185] with respect to the tendon compliance of the patella tendon, in that dynamometry, EMG and ultrasonography were utilised as well as the force-displacement relationship to calculate tendon mechanical stiffness. Therefore, an accurate, reproducible and non-invasive assessment of tendon properties *in vivo* is required to maximise the ability to detect a genetic contribution to the interindividual variability in mechanical properties of human tendon.

From a genetic perspective, a powerful approach to find significant DNA polymorphisms associated with tendon phenotypes could be to perform genome-wide association studies (GWAS). Geneticists have developed genotyping arrays (often called SNP chips) that can now assay up to ~2 million variants simultaneously which, due to linkage disequilibrium, capture a substantial proportion of total genomic variability [186]. GWAS involves testing a comprehensive catalogue of common genetic variants, and can be applied to a case-control study (to find those variants associated with a medical condition or other extreme phenotype, such as patients with tendinopathies or athletes successful in sports requiring high flexibility) or to a genotype-phenotype association study (to find those variants associated with a phenotype measured on a continuous scale). By testing all common variants, one could pinpoint key genes and shed light on underlying mechanisms. Three key results have emerged from GWAS: (1) most traits can be influenced by a large number of loci; (2) the vast majority of the common variants at these loci have a moderate effect, increasing risk by 10–50% (similar to effects of many environmental risk factors); and (3) the loci include most of the genes found by linkage analysis, but reveal many more genes not previously implicated [187]. Over the next decade, it would be desirable to conduct genetic studies of thousands of patients with tendinopathies (in case-control studies) and of healthy subjects with measures of flexibility (genotype-phenotype studies), with appropriate combinations of GWAS and sequencing. In turn, intensive functional studies will be required to characterize the genes and pathways, and to construct animal models that mimic human tendon physiology.

#### ACKNOWLEDGEMENTS

This research has been supported in part by an International Joint Project grant from the Royal Society, London, UK.

#### CONFLICT OF INTEREST

Declared none.

#### REFERENCES

- [1] Neuberger A, Perrone JC, Slack HG. The relative metabolic inertia of tendon collagen in the rat. *Biochem J* 1951; 49: 199-204.
- [2] Peacock EE Jr. Biology of tendon repair. *N Engl J Med* 1967; 276: 680-3.
- [3] McGinnis PM. Biomechanics of sport and exercise. 2nd ed. Champaign, IL ; London: Human Kinetics 2005.
- [4] Wilson GJ, Murphy AJ, Pryor JF. Musculotendinous stiffness: its relationship to eccentric, isometric, and concentric performance. *J Appl Physiol* 1994; 76: 2714-9.
- [5] Wilson GJ, Elliott BC, Wood GA. Stretch shorten cycle performance enhancement through flexibility training. *Med Sci Sports Exerc* 1992; 24: 116-23.
- [6] Walshe AD, Wilson GJ. The influence of musculotendinous stiffness on drop jump performance. *Can J Appl Physiol* 1997; 22: 117-32.
- [7] Lichtwark GA, Barclay CJ. The influence of tendon compliance on muscle power output and efficiency during cyclic contractions. *J Exp Biol* 2010; 213: 707-14.
- [8] Lichtwark GA, Wilson AM. Optimal muscle fascicle length and tendon stiffness for maximising gastrocnemius efficiency during human walking and running. *J Theor Biol* 2008; 252: 662-73.
- [9] Arnoczky SP, Lavagnino M, Egerbacher M, *et al.* Loss of homeostatic strain alters mechanostat "set point" of tendon cells *in vitro*. *Clin Orthop Relat Res* 2008; 466: 1583-91.
- [10] Ansoorge HL, Beredjikian PK, Soslowsky LJ. CD44 deficiency improves healing tendon mechanics and increases matrix and cytokine expression in a mouse patellar tendon injury model. *J Orthop Res* 2009; 27: 1386-91.
- [11] Lavagnino M, Arnoczky SP, Kepich E, *et al.* A finite element model predicts the mechanotransduction response of tendon cells to cyclic tensile loading. *Biomech Model Mechanobiol* 2008; 7: 405-16.
- [12] Arampatzis A, Karamanidis K, Albracht K. Adaptational responses of the human Achilles tendon by modulation of the applied cyclic strain magnitude. *J Exp Biol* 2007; 210: 2743-53.
- [13] Maeda E, Shelton JC, Bader DL, *et al.* Differential regulation of gene expression in isolated tendon fascicles exposed to cyclic tensile strain *in vitro*. *J Appl Physiol* 2009; 106: 506-12.
- [14] Kjaer M, Langberg H, Heinemeier K, *et al.* From mechanical loading to collagen synthesis, structural changes and function in human tendon. *Scand J Med Sci Sports* 2009; 19: 500-10.
- [15] Coupe C, Kongsgaard M, Aagaard P, *et al.* Habitual loading results in tendon hypertrophy and increased stiffness of the human patellar tendon. *J Appl Physiol* 2008; 105: 805-10.
- [16] Maffulli N, Khan KM, Puddu G. Overuse tendon conditions: time to change a confusing terminology. *Arthroscopy* 1998; 14: 840-3.
- [17] Khan KM, Cook JL, Bonar F, *et al.* Histopathology of common tendinopathies. Update and implications for clinical management. *Sports Med* 1999; 27: 393-408.
- [18] Devitt D, Koike Y, Doherty GP, *et al.* The ability of ultrasonography, magnetic resonance imaging and bone mineral densitometry to predict the strength of human Achilles' tendons. *Arch Phys Med Rehabil* 2009; 90: 756-60.
- [19] Kongsgaard M, Aagaard P, Kjaer M, *et al.* Structural Achilles tendon properties in athletes subjected to different exercise modes and in Achilles tendon rupture patients. *J Appl Physiol* 2005; 99: 1965-71.
- [20] Kongsgaard M, Kovanen V, Aagaard P, *et al.* Corticosteroid injections, eccentric decline squat training and heavy slow resistance training in patellar tendinopathy. *Scand J Med Sci Sports* 2009; 19: 790-802.
- [21] Kongsgaard M, Qvortrup K, Larsen J, *et al.* Fibril Morphology and Tendon Mechanical Properties in Patellar Tendinopathy. *Am J Sports Med* 2010; 38: 749-56.
- [22] Lake SP, Miller KS, Elliott DM, *et al.* Effect of fiber distribution and realignment on the nonlinear and inhomogeneous mechanical properties of human supraspinatus tendon under longitudinal tensile loading. *J Orthop Res* 2009; 27: 1596-602.
- [23] Sconfienza LM, Silvestri E, Cimmino MA. Sonoelastography in the evaluation of painful Achilles tendon in amateur athletes. *Clin Exp Rheumatol* 2010; 28: 373-8.
- [24] Liu H-Y, Boling M, Padua D, *et al.* *In vivo* evaluation of patellar tendon stiffness in individuals with patellofemoral pain syndrome. *Appl Bionics Biomech* 2008; 5: 59-63.
- [25] Arya S, Kulig K. Tendinopathy alters mechanical and material properties of the Achilles tendon. *J Appl Physiol* 2010; 108: 670-5.
- [26] Child S, Crossley K, Bryant A, *et al.* Stiffness of the human Achilles tendon is altered in people with Achilles tendinopathy. *Journal of Science and Medicine in Sport*. [doi: DOI: 10.1016/j.jsams.2008.12.047] 2009; 12: S18-S.
- [27] Riley GP, Harrall RL, Constant CR, *et al.* Tendon degeneration and chronic shoulder pain: changes in the collagen composition of the human rotator cuff tendons in rotator cuff tendinitis. *Ann Rheum Dis* 1994; 53: 359-66.
- [28] Samiric T, Parkinson J, Ilic MZ, *et al.* Changes in the composition of the extracellular matrix in patellar tendinopathy. *Matrix Biol* 2009; 28: 230-6.

- [29] Diamant J, Keller A, Baer E, *et al.* Collagen; ultrastructure and its relation to mechanical properties as a function of ageing. *Proc R Soc Lond B Biol Sci* 1972; 180: 293-315.
- [30] Bailey AJ, Paul RG, Knott L. Mechanisms of maturation and ageing of collagen. *Mech Ageing Dev* 1998; 106: 1-56.
- [31] Reeves ND, Maganaris CN, Narici MV. Effect of strength training on human patella tendon mechanical properties of older individuals. *J Physiol* 2003; 548: 971-81.
- [32] O'Brien TD, Reeves ND, Baltzopoulos V, *et al.* Mechanical properties of the patellar tendon in adults and children. *J Biomech* 2010; 43: 1190-5.
- [33] Kubo K, Kanehisa H, Fukunaga T. Gender differences in the viscoelastic properties of tendon structures. *Eur J Appl Physiol* 2003; 88: 520-6.
- [34] Magnusson SP, Hansen M, Langberg H, *et al.* The adaptability of tendon to loading differs in men and women. *Int J Exp Pathol* 2007; 88: 237-40.
- [35] Onambele GN, Burgess K, Pearson SJ. Gender-specific *in vivo* measurement of the structural and mechanical properties of the human patellar tendon. *J Orthop Res* 2007; 25: 1635-42.
- [36] Posthumus M, Collins M, Cook J, *et al.* Components of the transforming growth factor- $\beta$  family and the pathogenesis of human Achilles tendon pathology--a genetic association study. *Rheumatology (Oxford)* 2010; 49(11): 2090-7.
- [37] Mokone GG, Gajjar M, September AV, *et al.* The guanine-thymine dinucleotide repeat polymorphism within the tenascin-C gene is associated with achilles tendon injuries. *Am J Sports Med* 2005; 33: 1016-21.
- [38] September AV, Cook J, Handley CJ, *et al.* Variants within the COL5A1 gene are associated with achilles tendinopathy in two populations. *Br J Sports Med* 2008; 43(5): 357-65.
- [39] Mokone GG, Schweltnus MP, Noakes TD, *et al.* The COL5A1 gene and Achilles tendon pathology. *Scand J Med Sci Sports* 2006; 16: 19-26.
- [40] Raleigh SM, van der Merwe L, Ribbans WJ, *et al.* Variants within the MMP3 gene are associated with Achilles tendinopathy: possible interaction with the COL5A1 gene. *Br J Sport Med* 2009; 43: 514-20.
- [41] Collins M, Mokone GG, September AV, *et al.* The COL5A1 genotype is associated with range of motion measurements. *Scand J Med Sci Sports* 2009; 19(6): 803-10.
- [42] Brown JC, Miller CJ, Schweltnus MP, *et al.* Range of motion measurements diverge with increasing age for COL5A1 genotypes. *Scand J Med Sci Sports* 2011; 21(6): e266-72.
- [43] Posthumus M, September AV, Keegan M, *et al.* Genetic risk factors for anterior cruciate ligament ruptures: COL1A1 gene variant. *Br J Sports Med* 2009; 43: 352-6.
- [44] Khoschnau S, Melhus H, Jacobson A, *et al.* Type I collagen alpha 1 Sp1 polymorphism and the risk of cruciate ligament ruptures or shoulder dislocations. *Am J Sports Med* 2008; 36: 2432-6.
- [45] Chanut-Delalande H, Bonod-Bidaud C, Cogne S, *et al.* Development of a functional skin matrix requires deposition of collagen V heterotrimers. *Mol Cell Biol* 2004; 24: 6049-57.
- [46] Roulet M, Ruggiero F, Karsenty G, *et al.* A comprehensive study of the spatial and temporal expression of the col5a1 gene in mouse embryos: a clue for understanding collagen V function in developing connective tissues. *Cell Tissue Res* 2007; 327: 323-32.
- [47] Birney E, Andrews TD, Bevan P, *et al.* An Overview of Ensembl. *Genome Res* 2004; 14: 925-8.
- [48] Birk DE, Fitch JM, Babiarz JP, *et al.* Collagen type I and type V are present in the same fibril in the avian corneal stroma. *J Cell Biol* 1988; 106: 999-1008.
- [49] Birk DE, Fitch JM, Babiarz JP, *et al.* Collagen fibrillogenesis *in vitro*: interaction of types I and V collagen regulates fibril diameter. *J Cell Sci* 1990; 95(Pt 4): 649-57.
- [50] Linsenmayer TF, Fitch JM, Birk DE. Heterotypic collagen fibrils and stabilizing collagens. Controlling elements in corneal morphogenesis? *Ann N Y Acad Sci* 1990; 580: 143-60.
- [51] Marchant JK, Hahn RA, Linsenmayer TF, *et al.* Reduction of type V collagen using a dominant-negative strategy alters the regulation of fibrillogenesis and results in the loss of corneal-specific fibril morphology. *J Cell Biol* 1996; 135: 1415-26.
- [52] Niyibizi C, Eyre DR. Structural analysis of the extension peptides on matrix forms of type V collagen in fetal calf bone and skin. *Biochim Biophys Acta* 1993; 1203: 304-9.
- [53] Dressler MR, Butler DL, Wenstrup R, *et al.* A potential mechanism for age-related declines in patellar tendon biomechanics. *J Orthop Res* 2002; 20: 1315-22.
- [54] Birch HL. Tendon matrix composition and turnover in relation to functional requirements. *Int J Exp Pathol* 2007; 88: 241-8.
- [55] Hansen M, Kongsgaard M, Holm L, *et al.* Effect of estrogen on tendon collagen synthesis, tendon structural characteristics, and biomechanical properties in postmenopausal women. *J Appl Physiol* 2009; 106: 1385-93.
- [56] Eliasson P, Fahlgren A, Pasternak B, *et al.* Unloaded rat Achilles tendons continue to grow, but lose viscoelasticity. *J Appl Physiol* 2007; 103: 459-63.
- [57] Reddy GK. Cross-linking in collagen by nonenzymatic glycation increases the matrix stiffness in rabbit achilles tendon. *Exp Diabetes Res* 2004; 5: 143-53.
- [58] Maruhashi T, Kii I, Saito M, *et al.* Interaction between Periostin and BMP-1 Promotes Proteolytic Activation of Lysyl Oxidase. *J Biol Chem* 2010; 285: 13294-303.
- [59] Hansen P, Hassenkam T, Svensson RB, *et al.* Glutaraldehyde cross-linking of tendon--mechanical effects at the level of the tendon fascicle and fibril. *Connect Tissue Res* 2009; 50: 211-22.
- [60] Menard D, Stanish WD. The aging athlete. *Am J Sports Med* 1989; 17: 187-96.
- [61] Viidik A. Connective tissues--possible implications of the temporal changes for the aging process. *Mech Ageing Dev* 1979; 9: 267-85.
- [62] Birk DE. Type V collagen: heterotypic type I/V collagen interactions in the regulation of fibril assembly. *Micron* 2001; 32: 223-37.
- [63] Wenstrup RJ, Florer JB, Brunskill EW, *et al.* Type V collagen controls the initiation of collagen fibril assembly. *J Biol Chem* 2004; 279: 53331-7.
- [64] Jozsa L, Balint JB, Kannus P, *et al.* Distribution of blood groups in patients with tendon rupture. An analysis of 832 cases. *J Bone Joint Surg Br* 1989; 71: 272-4.
- [65] Kujala UM, Jarvinen M, Natri A, *et al.* ABO blood groups and musculoskeletal injuries. *Injury* 1992; 23: 131-3.
- [66] Magra M, Maffulli N. Genetic aspects of tendinopathy. *J Sci Med Sport* 2008; 11: 243-7.
- [67] Ambros V. The functions of animal microRNAs. *Nature* 2004; 431: 350-5.
- [68] Carthew RW. Gene regulation by microRNAs. *Curr Opin Genet Dev* 2006; 16: 203-8.
- [69] Witvrouw E, Danneels L, Asselman P, *et al.* Muscle Flexibility as a Risk Factor for Developing Muscle Injuries in Male Professional Soccer Players. *Am J Sports Med* 2003; 31: 41-6.
- [70] Witvrouw E, Bellemans J, Lysens R, *et al.* Intrinsic Risk Factors for the Development of Patellar Tendinitis in an Athletic Population. *Am J Sports Med* 2001; 29: 190-5.
- [71] Battie MC, Levalahti E, Videman T, *et al.* Heritability of lumbar flexibility and the role of disc degeneration and body weight. *J Appl Physiol* 2008; 104: 379-85.
- [72] Hakim AJ, Cherkas LF, Grahame R, *et al.* The genetic epidemiology of joint hypermobility: a population study of female twins. *Arthritis Rheum* 2004; 50: 2640-4.
- [73] Maes HH, Beunen GP, Vlietinck RF, *et al.* Inheritance of physical fitness in 10-yr-old twins and their parents. *Med Sci Sports Exerc* 1996; 28: 1479-91.
- [74] Chatterjee S, Das N. Physical and motor fitness in twins. *Jpn J Physiol* 1995; 45: 519-34.
- [75] Malfait F, Paeppe Ad. Molecular genetics in <I>classic</I> Ehlers-Danlos syndrome. *Am J Med Genet Part C: Seminars in Medical Genetics* 2005; 139C: 17-23.
- [76] Malfait F, Coucke P, Symoens S, *et al.* The molecular basis of classic Ehlers-Danlos syndrome: A comprehensive study of biochemical and molecular findings in 48 unrelated patients. *Hum Mutat* 2005; 25: 28-37.
- [77] Mitchell AL, Schwarze U, Jennings JF, *et al.* Molecular mechanisms of classical Ehlers-Danlos syndrome (EDS). *Hum Mutat* 2009; 30: 995-1002.
- [78] Schwarze U, Atkinson M, Hoffman GG, *et al.* Null alleles of the COL5A1 gene of type V collagen are a cause of the classical forms of Ehlers-Danlos syndrome (types I and II). *Am J Hum Genet* 2000; 66: 1757-65.
- [79] Vogel A, Holbrook KA, Steinmann B, *et al.* Abnormal collagen fibril structure in the gravis form (type I) of Ehlers-Danlos syndrome. *Lab Invest* 1979; 40: 201-6.

- [80] Goeken LN, Hof AL. Instrumental straight-leg raising: results in healthy subjects. *Arch Phys Med Rehabil* 1993; 74: 194-203.
- [81] ACSM's resource manual for Guidelines for exercise testing and prescription. 5th ed. ed. Kaminsky LA, editor. Baltimore, MD: Lippincott Williams & Wilkins 2006.
- [82] Goeken LN, Hof AL. Instrumental straight-leg raising: a new approach to Lasegue's test. *Arch Phys Med Rehabil* 1991; 72: 959-66.
- [83] Goeken LN, Hof AL. Instrumental straight-leg raising: results in patients. *Arch Phys Med Rehabil* 1994; 75: 470-7.
- [84] McHugh MP, Kremenich IJ, Fox MB, *et al.* The role of mechanical and neural restraints to joint range of motion during passive stretch. *Med Sci Sports Exerc* 1998; 30: 928-32.
- [85] Fernandez JE, Stubbs NB. Mathematical modeling and testing of the sit and reach test. *Int J Ind Ergonom* 1989; 3: 201-5.
- [86] Kubo K, Kanehisa H, Fukunaga T. Is passive stiffness in human muscles related to the elasticity of tendon structures? *Eur J Appl Physiol* 2001; 85: 226-32.
- [87] Burgess KE, Pearson SJ, Onambele GL. Patellar tendon properties with fluctuating menstrual cycle hormones. *J Strength Cond Res* 2010; 24: 2088-95.
- [88] Kubo K, Kanehisa H, Fukunaga T. Effects of different duration isometric contractions on tendon elasticity in human quadriceps muscles. *J Physiol* 2001; 536: 649-55.
- [89] Kubo K, Akima H, Kouzaki M, *et al.* Changes in the elastic properties of tendon structures following 20 days bed-rest in humans. *Eur J Appl Physiol* 2000; 83: 463-8.
- [90] Reeves ND, Maganaris CN, Ferretti G, *et al.* Influence of 90-day simulated microgravity on human tendon mechanical properties and the effect of resistive countermeasures. *J Appl Physiol* 2005; 98: 2278-86.
- [91] Erickson HP. Tenascin-C, tenascin-R and tenascin-X: a family of talented proteins in search of functions. *Curr Opin Cell Biol* 1993; 5: 869-76.
- [92] Jarvinen TA, Jozsa L, Kannus P, *et al.* Mechanical loading regulates the expression of tenascin-C in the myotendinous junction and tendon but does not induce de novo synthesis in the skeletal muscle. *J Cell Sci* 2003; 116: 857-66.
- [93] Jarvinen TA, Jozsa L, Kannus P, *et al.* Mechanical loading regulates tenascin-C expression in the osteotendinous junction. *J Cell Sci* 1999; 112 (Pt 18): 3157-66.
- [94] Sarasa-Renedo A, Chiquet M. Mechanical signals regulating extracellular matrix gene expression in fibroblasts. *Scand J Med Sci Sports* 2005; 15: 223-30.
- [95] Jarvinen TA, Kannus P, Jarvinen TL, *et al.* Tenascin-C in the pathobiology and healing process of musculoskeletal tissue injury. *Scand J Med Sci Sports* 2000; 10: 376-82.
- [96] Oberhauser AF, Marszalek PE, Erickson HP, *et al.* The molecular elasticity of the extracellular matrix protein tenascin. *Nature* 1998; 393: 181-5.
- [97] Eliasson P, Andersson T, Aspenberg P. Rat Achilles tendon healing: mechanical loading and gene expression. *J Appl Physiol* 2009; 107: 399-407.
- [98] Chiquet M. Regulation of extracellular matrix gene expression by mechanical stress. *Matrix Biol* 1999; 18: 417-26.
- [99] Chiquet-Ehrismann R, Tucker RP. Connective tissues: signalling by tenascins. *Int J Biochem Cell Biol* 2004; 36: 1085-9.
- [100] Ireland D, Harrall R, Curry V, *et al.* Multiple changes in gene expression in chronic human Achilles tendinopathy. *Matrix Biol* 2001; 20: 159-69.
- [101] Riley GP, Harrall RL, Cawston TE, *et al.* Tenascin-C and human tendon degeneration. *Am J Pathol* 1996; 149: 933-43.
- [102] Kaariainen M, Jarvinen T, Jarvinen M, *et al.* Relation between myofibers and connective tissue during muscle injury repair. *Scand J Med Sci Sports* 2000; 10: 332-7.
- [103] Kaariainen M, Kaariainen J, Jarvinen TL, *et al.* Integrin and dystrophin associated adhesion protein complexes during regeneration of shearing-type muscle injury. *Neuromuscul Disord* 2000; 10: 121-32.
- [104] Onambele GL, Narici MV, Maganaris CN. Calf muscle-tendon properties and postural balance in old age. *J Appl Physiol* 2006; 100: 2048-56.
- [105] Chiquet-Ehrismann R, Chiquet M. Tenascins: regulation and putative functions during pathological stress. *J Pathol* 2003; 200: 488-99.
- [106] Jones FS, Jones PL. The tenascin family of ECM glycoproteins: structure, function, and regulation during embryonic development and tissue remodeling. *Dev Dyn* 2000; 218: 235-59.
- [107] Jones PL, Jones FS. Tenascin-C in development and disease: gene regulation and cell function. *Matrix Biol* 2000; 19: 581-96.
- [108] Murphy G, Knauper V. Relating matrix metalloproteinase structure to function: why the "hemopexin" domain? *Matrix Biol* 1997; 15: 511-8.
- [109] Sternlicht MD, Werb Z. How matrix metalloproteinases regulate cell behavior. *Annu Rev Cell Dev Biol* 2001; 17: 463-516.
- [110] Somerville RP, Oblander SA, Apte SS. Matrix metalloproteinases: old dogs with new tricks. *Genome Biol* 2003; 4: 216.
- [111] Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. *Circ Res* 2003; 92: 827-39.
- [112] Matrisian LM. Metalloproteinases and their inhibitors in matrix remodeling. *Trends Genet* 1990; 6: 121-5.
- [113] Riley G. The pathogenesis of tendinopathy. A molecular perspective. *Rheumatology* 2004; 43: 131-42.
- [114] Tsuzaki M, Bynum D, Almekinders L, *et al.* ATP modulates load-inducible IL-1beta, COX 2, and MMP-3 gene expression in human tendon cells. *J Cell Biochem* 2003; 89: 556-62.
- [115] Archambault J, Tsuzaki M, Herzog W, *et al.* Stretch and interleukin-1beta induce matrix metalloproteinases in rabbit tendon cells *in vitro*. *J Orthop Res* 2002; 20: 36-9.
- [116] Sun HB, Andarawis-Puri N, Li Y, *et al.* Cycle-dependent matrix remodeling gene expression response in fatigue-loaded rat patellar tendons. *J Orthop Res* 2010; 28: 1380-6.
- [117] Jones GC, Corps AN, Pennington CJ, *et al.* Expression profiling of metalloproteinases and tissue inhibitors of metalloproteinases in normal and degenerate human achilles tendon. *Arthritis Rheum* 2006; 54: 832-42.
- [118] Asundi KR, Rempel DM. Cyclic loading inhibits expression of MMP-3 but not MMP-1 in an *in vitro* rabbit flexor tendon model. *Clin Biomech (Bristol, Avon)* 2008; 23: 117-21.
- [119] Birch HL, Wilson AM, Goodship AE. Physical activity: does long-term, high-intensity exercise in horses result in tendon degeneration? *J Appl Physiol* 2008; 105: 1927-33.
- [120] Thornton GM, Shao X, Chung M, *et al.* Changes in mechanical loading lead to tendonspecific alterations in MMP and TIMP expression: influence of stress deprivation and intermittent cyclic hydrostatic compression on rat supraspinatus and Achilles tendons. *Br J Sports Med* 2010; 44: 698-703.
- [121] Leigh DR, Abreu EL, Derwin KA. Changes in gene expression of individual matrix metalloproteinases differ in response to mechanical unloading of tendon fascicles in explant culture. *J Orthop Res* 2008; 26: 1306-12.
- [122] Lo IK, Marchuk LL, Leatherbarrow KE, *et al.* Collagen fibrillogenesis and mRNA levels in the maturing rabbit medial collateral ligament and patellar tendon. *Connect Tissue Res* 2004; 45: 11-22.
- [123] Parkinson J, Samiric T, Ilic MZ, *et al.* Changes in proteoglycan metabolism is a characteristic of human patellar tendinopathy. *Arthritis Rheum* 2010; 62(10): 3028-35.
- [124] de Mos M, van El B, DeGroot J, *et al.* Achilles Tendinosis. *Am J Sports Med* 2007; 35: 1549-56.
- [125] Riley GP, Curry V, DeGroot J, *et al.* Matrix metalloproteinase activities and their relationship with collagen remodelling in tendon pathology. *Matrix Biol* 2002; 21: 185-95.
- [126] Sullivan BE, Carroll CC, Jemiolo B, *et al.* Effect of acute resistance exercise and sex on human patellar tendon structural and regulatory mRNA expression. *J Appl Physiol* 2009; 106: 468-75.
- [127] Gray J, Taunton JE, McKenzie DC, *et al.* A Survey of Injuries to the Anterior Cruciate Ligament of the Knee in Female Basketball Players. *Int J Sports Med* 1985; 06: 314,6.
- [128] Koch W, de Waha A, Hoppmann P, *et al.* Haplotypes and 5A/6A polymorphism of the matrix metalloproteinase-3 gene in coronary disease: case-control study and a meta-analysis. *Atherosclerosis* 2010; 208: 171-6.
- [129] Beyzade S, Zhang S, Wong YK, *et al.* Influences of matrix metalloproteinase-3 gene variation on extent of coronary atherosclerosis and risk of myocardial infarction. *J Am Coll Cardiol* 2003; 41: 2130-7.
- [130] Ye S, Patodi N, Walker-Bone K, *et al.* Variation in the matrix metalloproteinase-3, -7, -12 and -13 genes is associated with

- functional status in rheumatoid arthritis. *Int J Immunogenet* 2007; 34: 81-5.
- [131] Samnegard A, Silveira A, Lundman P, *et al.* Serum matrix metalloproteinase-3 concentration is influenced by MMP-3 -1612 5A/6A promoter genotype and associated with myocardial infarction. *J Int Med* 2005; 258: 411-9.
- [132] Posthumus M, Raleigh SM, Ribbans WJ, *et al.* A functional variant within the MMP3 gene does not associate with human range of motion. *J Sci Med Sport* 2010; 13(6): 630-2.
- [133] Witvrouw E, Mahieu N, Roosen P, *et al.* The role of stretching in tendon injuries. *Br J Sports Med* 2007; 41: 224-6.
- [134] Schreuder H, Liesum A, Pohl J, *et al.* Crystal structure of recombinant human growth and differentiation factor 5: evidence for interaction of the type I and type II receptor-binding sites. *Biochem Biophys Res Commun* 2005; 329: 1076-86.
- [135] Eliasson P, Fahlgren A, Aspenberg P. Mechanical load and BMP signaling during tendon repair: a role for follistatin? *Clin Orthop Relat Res* 2008; 466: 1592-7.
- [136] Moore YR, Dickinson DP, Wikesjo UM. Growth/differentiation factor-5: a candidate therapeutic agent for periodontal regeneration? A review of pre-clinical data. *J Clin Periodontol* 2010; 37: 288-98.
- [137] Alexander TH, Sage AB, Chen AC, *et al.* Insulin-like Growth Factor-I and Growth Differentiation Factor-5 Promote the Formation of Tissue-Engineered Human Nasal Septal Cartilage. *Tissue Eng Part C Methods* 2010; 16(5): 1213-21.
- [138] Wolfman NM, Hattersley G, Cox K, *et al.* Ectopic induction of tendon and ligament in rats by growth and differentiation factors 5, 6, and 7, members of the TGF-beta gene family. *J Clin Invest* 1997; 100: 321-30.
- [139] Aspenberg P, Forslund C. Enhanced tendon healing with GDF 5 and 6. *Acta Orthop Scand* 1999; 70: 51-4.
- [140] Mikic B, Schalet BJ, Clark RT, *et al.* GDF-5 deficiency in mice alters the ultrastructure, mechanical properties and composition of the Achilles tendon. *J Orthop Res* 2001; 19: 365-71.
- [141] Clark RT, Johnson TL, Schalet BJ, *et al.* GDF-5 deficiency in mice leads to disruption of tail tendon form and function. *Connect Tissue Res* 2001; 42: 175-86.
- [142] Chhabra A, Tsou D, Clark RT, *et al.* GDF-5 deficiency in mice delays Achilles tendon healing. *J Orthop Res* 2003; 21: 826-35.
- [143] Loiselle AE, Bragdon GA, Jacobson JA, *et al.* Remodeling of murine intrasynovial tendon adhesions following injury: MMP and neontendon gene expression. *J Orthop Res* 2009; 27: 833-40.
- [144] Rickert M. BMP-14 gene therapy increases tendon tensile strength in a rat model of achilles tendon injury. *J Bone Joint Surg Am* 2008; 90: 445.
- [145] Dines JS, Weber L, Razzano P, *et al.* The effect of growth differentiation factor-5-coated sutures on tendon repair in a rat model. *J Shoulder Elbow Surg* 2007; 16: S215-21.
- [146] Bolt P, Clerk AN, Luu HH, *et al.* BMP-14 gene therapy increases tendon tensile strength in a rat model of Achilles tendon injury. *J Bone Joint Surg Am* 2007; 89: 1315-20.
- [147] Hogan M, Girish K, James R, *et al.* Growth differentiation factor-5 regulation of extracellular matrix gene expression in murine tendon fibroblasts. *J Tissue Eng Regen Med* 2011; 5(3): 191-200.
- [148] Henn RF, Christina EK, Michael WK, *et al.* Augmentation of Zone II Flexor Tendon Repair Using Growth Differentiation Factor 5 in a Rabbit Model. *J Hand Surg Am* 2010; 35: 1825-32.
- [149] Keller TC, Hogan MV, Kesturu G, *et al.* Growth/differentiation factor-5 modulates the synthesis and expression of extracellular matrix and cell-adhesion-related molecules of rat Achilles tendon fibroblasts. *Connect Tissue Res* 2011; 52(4): 353-64.
- [150] Douzgou S, Lehmann K, Mingarelli R, *et al.* Compound heterozygosity for GDF5 in Du Pan type chondrodysplasia. *Am J Med Genet A* 2008; 146A: 2116-21.
- [151] Faiyaz-UI-Haque M, Faqih EA, Al-Zaidan H, *et al.* Grebe-type chondrodysplasia: a novel missense mutation in a conserved cysteine of the growth differentiation factor 5. *J Bone Miner Metab* 2008; 26: 648-52.
- [152] Schwabe GC, Turkmen S, Leschik G, *et al.* Brachydactyly type C caused by a homozygous missense mutation in the prodomain of CDMP1. *Am J Med Genet A* 2004; 124A: 356-63.
- [153] Chapman K, Takahashi A, Meulenbelt I, *et al.* A meta-analysis of European and Asian cohorts reveals a global role of a functional SNP in the 5' UTR of GDF5 with osteoarthritis susceptibility. *Hum Mol Genet* 2008; 17: 1497-504.
- [154] Egli RJ, Southam L, Wilkins JM, *et al.* Functional analysis of the osteoarthritis susceptibility-associated GDF5 regulatory polymorphism. *Arthritis Rheum* 2009; 60: 2055-64.
- [155] Waarsing J, Kloppenburg M, Slagboom P, *et al.* Osteoarthritis susceptibility genes influence the association between hip morphology and osteoarthritis. *Arthritis Rheum* 2011; 63(5): 1349-54.
- [156] Ji J, Dai J, Shi D, *et al.* [Association of genetic and mechanical factors with age of onset of knee osteoarthritis]. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 2010; 27: 672-4.
- [157] Rouault K, Scotet V, Autret S, *et al.* Evidence of association between GDF5 polymorphisms and congenital dislocation of the hip in a Caucasian population. *Osteoarthritis Cartilage* 2010; 18(9): 1144-9.
- [158] Vaes RB, Rivadeneira F, Kerkhof JM, *et al.* Genetic variation in the GDF5 region is associated with osteoarthritis, height, hip axis length and fracture risk: the Rotterdam study. *Ann Rheum Dis* 2009; 68: 1754-60.
- [159] Sanna S, Jackson AU, Nagaraja R, *et al.* Common variants in the GDF5-UQCC region are associated with variation in human height. *Nat Genet* 2008; 40: 198-203.
- [160] Williams FM, Popham M, Hart DJ, *et al.* GDF5 single-nucleotide polymorphism rs143383 is associated with lumbar disc degeneration in Northern European women. *Arthritis Rheum* 2011; 63: 708-12.
- [161] Southam L, Rodriguez-Lopez J, Wilkins JM, *et al.* An SNP in the 5'-UTR of GDF5 is associated with osteoarthritis susceptibility in Europeans and with *in vivo* differences in allelic expression in articular cartilage. *Hum Mol Genet* 2007; 16: 2226-32.
- [162] Miyamoto Y, Mabuchi A, Shi D, *et al.* A functional polymorphism in the 5' UTR of GDF5 is associated with susceptibility to osteoarthritis. *Nat Genet* 2007; 39: 529-33.
- [163] Valdes AM, Evangelou E, Kerkhof HJ, *et al.* The GDF5 rs143383 polymorphism is associated with osteoarthritis of the knee with genome-wide statistical significance. *Ann Rheum Dis* 2010; 70(5): 873-5.
- [164] Maruhashi T, Kii I, Saito M, *et al.* Interaction between periostin and BMP-1 promotes proteolytic activation of lysyl oxidase. *J Biol Chem* 2010; 285: 13294-303.
- [165] Elliott DH. Structure and Function of Mammalian Tendon. *Biol Rev Camb Philos Soc* 1965; 40: 392-421.
- [166] Lemoine JK, Lee JD, Trappe TA. Impact of sex and chronic resistance training on human patellar tendon dry mass, collagen content, and collagen cross-linking. *Am J Physiol Regul Integr Comp Physiol* 2009; 296: R119-24.
- [167] Silver FH, Christiansen DL, Snowhill PB, *et al.* Transition from viscous to elastic-based dependency of mechanical properties of self-assembled type I collagen fibers. *J Appl Polym Sci* 2001; 79: 134-42.
- [168] Silver FH, Freeman JW, Seehra GP. Collagen self-assembly and the development of tendon mechanical properties. *J Biomech* 2003; 36: 1529-53.
- [169] Lian K, Zmuda JM, Nevitt MC, *et al.* Type I collagen alpha1 Sp1 transcription factor binding site polymorphism is associated with reduced risk of hip osteoarthritis defined by severe joint space narrowing in elderly women. *Arthritis Rheum* 2005; 52: 1431-6.
- [170] Kuivaniemi H, Tromp G, Prockop DJ. Mutations in fibrillar collagens (types I, II, III, and XI), fibril-associated collagen (type IX), and network-forming collagen (type X) cause a spectrum of diseases of bone, cartilage, and blood vessels. *Hum Mutat* 1997; 9: 300-15.
- [171] Jin H, van't Hof RJ, Albagha OM, *et al.* Promoter and intron 1 polymorphisms of COL1A1 interact to regulate transcription and susceptibility to osteoporosis. *Hum Mol Genet* 2009; 18: 2729-38.
- [172] Liu PY, Lu Y, Long JR, *et al.* Common variants at the PCOL2 and Sp1 binding sites of the COL1A1 gene and their interactive effect influence bone mineral density in Caucasians. *J Med Genet* 2004; 41: 752-7.
- [173] McGuigan FE, Reid DM, Ralston SH. Susceptibility to osteoporotic fracture is determined by allelic variation at the Sp1 site, rather than other polymorphic sites at the COL1A1 locus. *Osteoporos Int* 2000; 11: 338-43.
- [174] Tran BN, Nguyen ND, Center JR, *et al.* Enhancement of absolute fracture risk prognosis with genetic marker: the collagen I alpha 1 gene. *Calcif Tissue Int* 2009; 85: 379-88.

- [175] Van Pottelbergh I, Goemaere S, Nuytinck L, *et al.* Association of the type I collagen alpha1 Sp1 polymorphism, bone density and upper limb muscle strength in community-dwelling elderly men. *Osteoporos Int* 2001; 12: 895-901.
- [176] Jin H, Evangelou E, Ioannidis JP, *et al.* Polymorphisms in the 5' flank of COL1A1 gene and osteoporosis: meta-analysis of published studies. *Osteoporos Int* 2011; 22: 911-21.
- [177] Hasegawa K. [The genetic basis for skeletal disease. Osteogenesis imperfecta and genetic abnormalities]. *Clin Calcium* 2010; 20: 1190-5.
- [178] Mann V, Hobson EE, Li B, *et al.* A COL1A1 Sp1 binding site polymorphism predisposes to osteoporotic fracture by affecting bone density and quality. *J Clin Invest* 2001; 107: 899-907.
- [179] Barbirato C, Almeida MG, Milanez M, *et al.* A novel COL1A1 gene-splicing mutation (c.1875+1G>C) in a Brazilian patient with osteogenesis imperfecta. *Genet Mol Res* 2009; 8: 173-8.
- [180] Trummer T, Brenner R, Just W, *et al.* Recurrent mutations in the COL1A2 gene in patients with osteogenesis imperfecta. *Clin Genet* 2001; 59: 338-43.
- [181] Garcia-Giralt N, Nogues X, Enjuanes A, *et al.* Two new single-nucleotide polymorphisms in the COL1A1 upstream regulatory region and their relationship to bone mineral density. *J Bone Miner Res* 2002; 17: 384-93.
- [182] Lippi G, Longo UG, Maffulli N. Genetics and sports. *Br Med Bull* 2010; 93: 27-47.
- [183] Parry DA. The molecular and fibrillar structure of collagen and its relationship to the mechanical properties of connective tissue. *Biophys Chem* 1988; 29: 195-209.
- [184] Morse CI, Degens H, Seynnes OR, *et al.* The acute effect of stretching on the passive stiffness of the human gastrocnemius muscle tendon unit. *J Physiol* 2008; 586: 97-106.
- [185] Pearson SJ, Onambele GN. Influence of time of day on tendon compliance and estimations of voluntary activation levels. *Muscle Nerve* 2006; 33: 792-800.
- [186] The International HapMap C. A haplotype map of the human genome. *Nature* 2005; 437: 1299-320.
- [187] Lander ES. Initial impact of the sequencing of the human genome. *Nature* 2011; 470: 187-97.

---

Received: November 14, 2011

Revised: January 23, 2012

Accepted: January 27, 2012

© Foster *et al.*; Licensee Bentham Open.

This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.