Tendon and Ligament Genetics: How Do They Contribute to Disease and Injury? A Narrative Review

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Abstract: A significant proportion of patients requiring musculoskeletal management present with tendon and ligament pathology. Our understanding of the intrinsic and extrinsic mechanisms that lead to such disabilities is increasing. However, the complexity underpinning these interactive multifactorial elements is still not fully characterised. Evidence highlighting the genetic components, either reducing or increasing susceptibility to injury, is increasing. This review examines the present understanding of the role genetic variations contribute to tendon and ligament injury risk. It examines the different elements of tendon and ligament structure and considers our knowledge of genetic influence on form, function, ability to withstand load, and undertake repair or regeneration. The role of epigenetic factors in modifying gene expression in these structures is also explored. It considers the challenges to interpreting present knowledge, the requirements, and likely pathways for future research, and whether such information has reached the point of clinical utility.

Keywords: genetics; epigenetics; tendon injury; ligament injury; tendinopathy

1. Introduction

It has been estimated that tendon and ligament injuries account for 30–50% of all sporting injuries [1]. The impact on people’s overall well-being, ability to work, and participate in exercise is significant. The health economic burden of managing such conditions is huge [2,3]. Is the prevalence of such injuries simply related to increased societal involvement in sport increasing exposure to extrinsic risk factors, such as running or other high impact activities? Alternatively, has the more sedentary lifestyle of many, combined with increasing levels of obesity, simply led to deconditioning and increased loading causing failure of these critical structures? How much does our inherited genotype influence our risk profile? This review considers the impact of genetic factors on the development of tendon and ligament injury and disease. What is our present state of knowledge of tendon and ligament structure and function? What do we understand about the influence of genetic factors and how might this knowledge be used in the future to reduce risk?

2. The Injury Causation Model and Jar Model

Tendon and ligament pathologies usually follow the injury causation model described by Meeuwisse in 1994 [4] and refined by Bittencourt in 2016 [5]. A complex interaction of intrinsic (including genetic factors) and extrinsic factors influences an individual’s specific profile along the ‘reduced to increased risk (predisposed) spectrum’. Following
an inciting episode, such as an injurious event, the problem becomes symptomatic to the patient (Figure 1). An injury may manifest itself as an acute episode or chronic condition. For the latter, there is often initial damage that is asymptomatic. Further subsequent inciting episodes, which can be identified by the patient, causes the damage to become symptomatic [6].

Figure 1. A hypothetical diagram showing the complex interaction of numerous genetic factors and extrinsic factors in determining an individual’s specific profile along the ‘reduced to increased risk (predisposed) spectrum’.

People inherit genetic factors which can result in an increased or reduced risk for many potential tendon and ligament injuries. However, the possession of such factors does not necessarily lead to the clinical development of such multifactorial conditions. Austin’s Jar Model for genetic risk was developed for use in psychiatric counselling [7] and applied to other areas of medicine, including cancers. It can be used in discussing risk for developing musculoskeletal damage (Figure 2).
Figure 2. The Jar Model. Accumulative risk factors for developing chronic tendon and ligament disease. Adapted from the ‘Jar Model’ of Jehannine Austin used in psychiatric genetic counselling (Austin, 2008) [7].

Extrinsic factors can be overwhelming, leading to injury such as in a tackle-playing sport causing ligament injury. However, for many instances of damage (usually sub-acute or chronic), both genetic and non-genetic intrinsic elements combine with extrinsic or environmental factors. Once the jar has ‘overflowed’, the damage becomes apparent. Careful review of modifiable risk factors (such as flexibility and body composition variables) combined with attempts to reduce their influence and the adoption of protective factors (such as improved nutrition and rest intervals) can improve tendon and ligament health. Medications such as steroids and certain fluoroquinolone antibiotics (examples include ciprofloxacin), can initiate tendon damage that can be ameliorated following their cessation [8,9].

3. Genetic Involvement in Musculoskeletal Condition

Most intrinsic risk factors for common tendon and ligament injuries have a genetic contribution [10]. For instance, flexibility has a heritability component estimated to between 64 and 70% [11,12]. Familial studies have reported a 40% heritability between twins for lateral epicondylitis [13], a five times increased risk for rotator cuff issues in siblings [14], and twin studies describing a knee anterior cruciate ligament (ACL) tear risk heritability of 69% [15].

Some rare orthopaedic conditions, such as pseudoachondroplasia and osteogenesis imperfecta, are caused by gene mutations [16,17]. However, most sports injuries are caused by extrinsic factors (such as training load) interacting with an individual’s genetic background and other intrinsic factors [18]. Additionally, some medical conditions with a
genetic background increase the risk of tendon pathology, including various seropositive and seronegative rheumatological conditions (such as gout and ankylosing spondylitis), Ehlers–Danlos syndrome, and other endocrine and metabolic disorders [19].

We note a spectrum of genetic-related connective tissue disorders. At one extreme are conditions associated with a classical Mendelian inheritance pattern—such as osteogenesis imperfecta [20], Ehlers–Danlos syndrome [21] and Marfan’s syndrome [22]. Establishing the genetic pattern of these conditions usually involves techniques such as linkage analysis or direct gene sequencing [23]. At the other end of the spectrum are conditions with a multifactorial aetiology often involving multiple and complex interactions between various genes with non-genetic intrinsic and extrinsic factors. These include gene–gene and gene with non-genetic factor interactions, making the investigation of the genetic contribution to such conditions more complicated.

4. Types of Genetic Studies

Depending on the prevalence of the phenotype and, therefore, the sample size, several study designs can be applied to investigate genetic influences on the development of multifactorial phenotypes such as tendon and ligament injuries.

4.1. Family Studies

The most basic of these formats are family studies. Disease inheritance patterns between family members indicate the degree of hereditability of such conditions. Examples of autosomal dominant (e.g., Huntington’s disease), autosomal recessive (e.g., phenylketonuria), and sex-linked related (e.g., Vitamin D resistant rickets with hypophosphatemia) are readily identified. However, the interactions of many factors (including genetic and non-genetic factors), such as a shared environmental load, influence the susceptibility to complex multifactorial clinical conditions such as tendon and ligament injuries. Therefore, identifying several members of a family with an identical injury is not common and, for this reason, a classical inheritance pattern is usually not identifiable. Twin studies represent a unique opportunity to identify the shared heritability component between twins together with a potential shared environmental exposure.

4.2. Case-Control Studies

Case-control genetic association studies allow interrogation and comparison of large data sets both within a population and between populations of different geographical locations and ancestry. This has been a popular method of identifying genetic risk factors of common multifactorial phenotypes including tendon and ligament pathologies [24]. However, it requires rigour in the phenotyping of cases and controls. Cases should be well defined, and diagnoses confirmed using preferably ‘gold standard’ methodology, such as imaging or surgical confirmation. Additionally, the multifactorial nature of the susceptibility, a comprehensive medical history, sporting history (including training regimes), medicines’ use, and familial injury history should be recorded for both cases and controls.

Controls’ selection is equally important, and individuals should be matched for sex, age, body mass index (BMI), and sports participation exposure and level, and any other potential confounders. Both the cases and controls may harbour genetic and non-genetic elements, which may confer an increased or decreased risk to sustaining a potential tendon or ligament injury. It is the balance of these multifactorial risk factors which will determine if the injury presents.

4.3. Hypothesis-Free Approach

Almost all past and current research has focused on the candidate gene approach where knowledge of a given gene and the injury is assumed (Section 4.2 above). There has been progress towards a hypothesis-free approach using the application of next generation sequencing technologies, such as genome wide association studies (GWAS). Most GWAS studies have used canine models [25–27] although one human study highlighted
three independent DNA sequence variants associated with ACL rupture—albeit of borderline significance [28]. A whole exome sequencing (WES) approach was also recently undertaken in a twin family study, where 11 novel variants were highlighted for further exploration in ACL injury susceptibility [29]. Recently, Gibbons used a hybrid approach of WES on targeted participants and applied a tiered filtering strategy to identify potentially biologically relevant new candidate-variants within previously implicated genes. This allowed further prioritisation in larger independent cohorts [30]. Currently, there are no whole genome sequencing datasets specific to exercise-related injury phenotypes, such as tendon and ligament injuries. In the future, genetic susceptibility would gain from research characterising the genome in relation to tendon and ligament injury.

5. Tendon: Structure, Function and Genetic Research

Tendons transfer the forces generated within muscles to their bone insertions. Tendons are composed of a heterogeneous population of tendon cells embedded within an extracellular matrix (ECM) consisting of collagen fibres, elastin fibres, proteoglycans, glycosaminoglycans, and glycoproteins.

5.1. Tendon Cells

Ninety to 95% of the cellular population within the mature tendon, which synthesise and regulate the components of the ECM, consist predominately of tenocytes and immature tenoblasts [31]. Tenocytes are predominately found within the fascicles between the collagen fibres and have an intricate network of connections using processes producing intercellular links via gap junctions [32]. The more rounded and metabolically active tenoblasts are primarily situated between fascicle units within the inter-fascicular matrix (IFM) [33,34]. Tendon cell activity alters with exposure to normal stresses, injury, and ageing. One to 4% of the cells within tendons are tendon stem/progenitor cells (TSPCs) which have similar characteristics to mesenchymal stem cells [35].

5.2. Collagen

Within the ECM, collagen is the major structural protein and constitutes 60–85% of the dry tendon weight. The collagen is arranged in a hierarchical manner [36] (Figure 3).
5.2.1. Type I Collagen

Type I collagen accounts for up to 90% of total collagen content. Type I collagen fibrils are the tendon’s primary structural elements. They provide tensile strength enhanced by cross-linking. Fibrils aggregate to fibres and, once again, fibres combine to form fascicles. Each fascicle is surrounded by an endotenon or IFM. Fascicles combine to form the tendon entity, which is bound together by a surrounding epitenon. A paratenon surrounds many tendons, such as the Achilles (Table 1).

**Table 1.** Principal tendon collagen types.

| Collagen Type | Functions | Associated Diseases |
|---------------|-----------|---------------------|
| Type I        | • Major tendon in collagen—90%  
• Primary structural elements | • Rare mutations within COL1A1 and COL1A2 genes, which encode for the α1 and α2 chains of type I collagen, respectively, cause osteogenesis imperfecta (OGI) [17] |
| Type III      | • Regulates type I collagen size (fibrillogenesis) [37]  
• Increases in ageing tendons [38]  
• Important role in flexibility and tissue strength  
• Active in early wound healing and, later, gradually replaced by type I collagen as the wound matures | • COL3A1 mutations have been found to cause vascular type of Ehlers–Danlos syndrome (EDS) [39] |
Table 1. Cont.

| Collagen Type | Functions | Associated Diseases |
|---------------|-----------|---------------------|
| Type V        | • Similar to type III  
                • Regulates fibril assembly and diameter and, thus, affects tendon mechanical properties [40]  
                • Content increases with age and in degenerative conditions [41] | • Common cause of EDS are mutations that inactivates one copy of COL5A1 (known as a haploinsufficiency) [39] |
| Type XI       | • Regulates type I and type II collagen fibrillogenesis by maintaining fibril spacing and diameter  
                • Although predominately expressed together with type II collagen, type XI collagen shares structural and functional homology with type V collagen and expressed in developing tendons [42]  
                • Proposed that genetic variants controlling type XI and V collagen production interact to regulate type I collagen fibril assembly [43] | • Mutations in type XI collagen genes (COL11A1 and COL11A2) associated with Stickler syndrome, Marshall syndrome, fibrochondrogenesis, otospondylometaepiphysial dysplasia deafness, and Weissenbacher–Zweymüller syndrome [44] |
| Types XII and XIV | • Type XII and XIV collagens are associated with type I collagen fibrils forming a molecular bridge between structural collagen and other ECM molecules [45]  
                        • Type XIV assists collagen fibrillogenesis regulation | Microdeletions on long arm of chromosome 6 containing COL12A1 gene cause developmental delay, mild dysmorphism and connective tissue laxity [46] |

Two studies, a South African cohort of Achilles tendon disease and a Turkish cohort of lateral epicondylitis of the elbow, investigated the COL1A1 rs1800012 functional DNA sequence variant with tendon disease and neither found an association [47,48]. However, later sub-analysis of acute Achilles tendon rupture in the South African cohort found that possession of a relatively rare TT genotype protects against injury [49]. More recently Gibbon [50] explored the COL1A1 rs1007946-rs1800012 haplotype and implicated G-T to be associated with reduced risk of Achilles tendinopathy and rupture (Table 2). Other collagen types, although in a minority in terms of mass, play an important part in determining collagen configuration, strength, and optimal function.

5.2.2. Type III Collagen

Type III collagen, encoded by COL3A1, is the second most common type comprising up to 10% of total collagen (Table 1). To date, no studies have investigated the association of COL3A1 variants with tendon injuries (although they have been undertaken in ligament injuries—see Section 6.1.3).

5.2.3. Type V Collagen

Type V collagen performs a similar role to Type III collagen. COL5A1 encodes for its α1 chain and is found close to the ABO gene on chromosome 9q34 (Table 1). A South African cohort study described four variants within the 3′-untranslated region (3′-UTR), which were independently associated with the development of chronic Achilles’ tendinopathy (AT). Additionally, a protective genotype for a single variant was identified [51]. The variants located within the 3′-UTR appear to influence the stability of the mRNA. This region appears to play an important role in post-transcriptional regulation [52,53]. The authors hypothesise that the C and T alleles of rs12722 influences COL5A1 mRNA stability and possibly through alternate mRNA structural forms, which may affect type V collagen expression. They further hypothesise that the mRNA stability is enhanced with the functional form associated with increased chronic AT, leading to increased type V collagen [54]. Collins further postulated on how increased type V collagen content was associated with altered mechanical properties of tendons and increased injury susceptibility [55]. A later study reported other genotypes, also within the functional COL5A1 3′-UTR, in South African and Australian populations that showed increased risk for chronic AT [56]. More recently, RNA
sequencing analyses in torn rotator cuff tissue vs. control tissue, showed that COL5A1 gene expression was markedly (3.01×) increased in tears, demonstrating its role in healing and/or remodelling [57].

The effect of altered genotypes in the MIR608 gene, which encodes a small micro-RNA (miRNA), was undertaken in Australian and South African groups [58]. An increased risk of AT was demonstrated with the rs4919510 CC genotype. This miRNA can bind to a recognition sequence within the COL5A1 and other genes 3'-UTR and inhibits translation. It was the first non-coding gene to be associated with soft-tissue injuries and increased understanding of the complex mechanisms involved in the regulation of type V collagen production. A GWAS study investigating a large cohort, including samples of different ancestry, suggested that MIR608 rs4919510 showed moderate evidence for AT susceptibility—although not at the level of significance required for a GWAS study [29] (Table 2).

Table 2. Summary of genetic research and tendon injuries: Collagen types I and V.

| Collagen Type | Reference | Country                      | Variant                        | Pathology                        | Subjects | Controls | Comments                                           |
|---------------|-----------|------------------------------|--------------------------------|-----------------------------------|----------|----------|---------------------------------------------------|
| Type I        | [46]      | South Africa                 | COL1A1 rs1800012               | Achilles’ tendinopathy and rupture | 126      | 125      | No significant association                        |
|               | [47]      | Turkey                       | COL1A1 rs1800012               | Lateral epicondylitis (common extensor origin) | 183      | 123      | No significant association                        |
|               | [58]      | Spain                        | COL1A1 rs1800012               | Patellar tendon injury           | 15       | 0        | No difference between severity of injury and gene variants. No controls were included |
|               | [48]      | South Africa and Sweden (controls only) | COL1A1 rs1800012 | Achilles’ tendon rupture | 41       | 581      | Significant association of the TT genotype protecting against acute Achilles’ tendon rupture |
|               | [49]      | South Africa and UK | COL1A1 rs1107946 rs1800012 | Achilles’ tendinopathy and rupture | 216      | 193      | Significant association of the (G-T) haplotype protecting against Achilles tendinopathy and rupture |
| Type V        | [50]      | South Africa                 | COL5A1 rs12722                | Achilles’ tendinopathy and rupture | 111      | 129      | Significant protective association with variant in tendon disease |
|               | [59]      | Italy                        | COL5A1 rs12722                | Bilateral quadriceps rupture     | 9        | 0        | No controls but association with a polymorphism and tendon rupture |
|               | [60]      | Italy                        | COL5A1 rs12722                | Bilateral quadriceps rupture     | 1        | 0        | No controls but association with a polymorphism and tendon rupture (same finding as Galasso) |
|               | [58]      | Spain                        | COL5A1 rs12722                | Patellar tendon injury           | 15       | 0        | No difference between severity of injury and gene variants. No controls were included |
|               | [55]      | South Africa, Australia      | COL5A1 rs12722 COL5A1 rs3196378 | Achilles’ tendinopathy and rupture | 178      | 342      | Significant protective association with variant of COL5A1 rs12722 in tendon disease In Australian cohort only, rs3196378 associated with tendinopathy |
|               | [57]      | South Africa, Australia      | COL5A1 3′-UTR rs71746744, rs1134170, rs16399 MIR608 rs4919510 | Achilles’ tendinopathy and rupture | 160      | 342      | All variants within the untranslated region of the COL5A1 gene and MIR variant were associated with Achilles tendon disease |

Green indicates an association and red indicates no association found.
5.2.4. Type XI Collagen

Type XI collagen is found in many structures including articular cartilage, bone, and muscle (Table 1). Variants in \textit{COL11A1} and \textit{COL11A2} interact with one another and with a \textit{COL5A1} 3'-UTR variant to modulate the AT risk in South African and Australian cohorts [43] (Table 3).

5.2.5. Types XII, XIV, and XXVII Collagens

None of the investigated functional variants of \textit{COL12A1} or \textit{COL14A1} which encode the \(\alpha\)-chains of types XII and XIV collagen have been associated with AT [59]. However, \textit{COL12A1} may be associated with acute Achilles tendon ruptures [59]. Saunders investigated several \textit{COL27A1} gene variants that encode Type XXVII collagen but could not identify any independent association with AT [60] (Tables 1 and 3).

5.3. Proteoglycans

Proteoglycans (PGs) represent up to 5% of tendon dry weight. They consist of a core protein attached to one or more glycosaminoglycans (GAGs). The GAG’s negative charge binds water, which makes up 55–70% of the total tendon weight. PGs are found between the ECM’s collagenous structural components (Table 4). There are no reported genetic studies associated with tendon PG. However, ligament PG genetic variants have been investigated (see Section 6.2.2).

5.4. Glycoproteins

Glycoproteins are a large family of structurally and functionally diverse proteins to which a carbohydrate group(s) is covalently attached. Two important tendinous glycoproteins are tenascin-C and cartilage oligomeric matrix protein (COMP) (Table 4).

**Table 3.** Summary of genetic research and tendon injuries: Collagen types XI, XII, XIV, and XXVII.

| Collagen Types | Reference | Country            | Variant                        | Pathology                  | Subjects | Controls | Comments                                                                                     |
|---------------|-----------|--------------------|-------------------------------|----------------------------|----------|----------|---------------------------------------------------------------------------------------------|
| Type XI       | [43]      | South Africa, Australia | \textit{COL11A1} rs1676456, rs3753841, \textit{COL11A2} rs1799907 | Achilles’ tendinopathy and rupture | 184      | 338      | No significant independent associations found  
|               |           |                    |                               |                            |          |          | Genes encoding structural and functionally related type XI (\textit{COL11A1} and \textit{COL11A2}) and type V (\textit{COL5A1}) collagens interact with one another to collectively modulate Achilles’ tendinopathy risk |
| Type XII      | [59]      | South Africa, Australia | \textit{COL12A1} rs240736, rs970547 | Achilles’ tendinopathy and rupture | 137      | 131      | No significant associations found  
|               |           |                    |                               |                            |          |          | There may be an association with Achilles tendon rupture                                      |
| Type XIV      | [59]      | South Africa, Australia | \textit{COL14A1} rs4870723, rs1563392 | Achilles’ tendinopathy and rupture | 137      | 131      | No significant associations found                                                                |
| Type XXVII    | [60]      | South Africa, Australia | \textit{COL27A1} (several polymorphisms) | Achilles’ tendinopathy and rupture | 178      | 340      | No significant associations found among variants                                           |

Green indicates an association and red indicates no association found.
Table 4. Regulatory and structural components of tendons.

| Component | Form and Types | Functions | Associated Diseases |
|-----------|----------------|-----------|---------------------|
| **Proteoglycans (PGs)** | - PGs: Found between extra-cellular matrix’s (ECM) collagenous structural components  
- PGs include Decorin, Lubricin, and Versican  
- Consist of a core protein attached to one or more glycosaminoglycans (GAGs) | - Decorin (80%): roles include collagen fibrillogenesis and potentially enhancing strength by facilitating load transfer between discontinuous collagen fibrils. Most abundant in tendinous areas most subject to tension  
- Lubricin: Found close to tendon periphery and aids tendon gliding with lubrication  
- Versican: Found within the interfascicular matrix (IFM) | - Mutations within TNX gene (a tenasin family member) causes an autosomal recessive form of EDS from tenasin-X deficiency [66] |
| **Glycoproteins** | - Tenascin-C (TNC): Protein is a hexamere, which binds to both ECM components and tenocyte surface receptors  
- Produced by TNC gene located close to the ABO gene on chromosome 9q34  
- TNC gene expression appears influenced by tension loading [61]  
- Important role in regulating the tenocytes need to interact with ECM components  
- Involved in cell adhesion and signalling, affecting cell proliferation and migration [62–64]  
- Appears to increase in the presence of tendon pathology [65]  
- Also known as thrombospondin V  
- Most abundant tendinous glycoprotein  
- Links to type I collagen and has five subunits allowing linkage between multiple fibrils  
- Found within the inter-fibrillar matrix [67] and tissues subject to significant loading, such as tendons, ligaments, cartilage, menisci, and intervertebral discs  
- Mediates cell-to-cell and cell-to-matrix interactions  
- Involved in cell-to-cell adhesion and ECM communication  
- Thrombospondin type 1 domain-containing protein 7A | - Pseudo-achondroplasia is caused by a heterozygous mutation in the gene encoding COMP [16]  
- A COMP gene mutation can cause multiple epiphyseal dysplasia (MED) |
| **Thrombospondins (THBS)** | - Family of glycoproteins performing dynamic role within ECM  
- Cartilage oligomeric matrix protein (COMP)  
- Also known as thrombospondin V  
- Most abundant tendinous glycoprotein  
- Links to type I collagen and has five subunits allowing linkage between multiple fibrils  
- Found within the inter-fibrillar matrix [67] and tissues subject to significant loading, such as tendons, ligaments, cartilage, menisci, and intervertebral discs  
- Mediates cell-to-cell and cell-to-matrix interactions  
- Involved in cell-to-cell adhesion and ECM communication  
- Thrombospondin type 1 domain-containing protein 7A | - Role debated  
- COMP-null knock out mice did not demonstrate any tendon abnormality [68]  
- Others reported in horses that COMP levels during growth correlate with mechanical strength at skeletal maturity [66]  
- COMP-null knock out mice did not demonstrate any tendon abnormality [68]  
- Others reported in horses that COMP levels during growth correlate with mechanical strength at skeletal maturity [66] | |
| **Elastin and Microfibrils** | - Elastin (ELN): Elastin provides elasticity to tendons, allowing them to stretch and return to their original state. Plays important load-bearing role in tendons and ligaments.  
- Microfibrils, such as the glycoprotein, Fibrillin:  
  - Found in ECM and become incorporated into insoluble microfibrils  
  - Plays a role in early elastogenesis acting as a scaffold for elastin deposition [73] | - E LN rs2071307 variant has been associated with other multifactorial ECM conditions, such as aortic stenosis [69] and aortic aneurysm [70]  
- E LN rs2071307 and FBN2 rs331079 variants associate with aortic and intracranial aneurysms, respectively, causing ECM disruption  
- Fibrillin-1 abnormalities have been associated with Marfan’s syndrome and Fibrillin-2 have with Beal’s syndrome or congenital contractural arachnodactyly [71,72] | |

[61, 62–64, 65, 66, 67, 68, 69, 70, 71, 72, 73]
5.4.1. Tenascin-C

Tenascin-C (TNC) plays an important role in regulating tenocytes that need to interact with ECM components (Table 4). The first research establishing a genetic association for a predisposition to Achilles tendon pathology was published in 2005 and implicated TNC [74]. A South African case-control association study reported that a Guanine-Thymine Dinucleotide repeat variant was associated with Achilles tendon injuries. A second study investigating different TNC variants (rs13321; rs2104772; rs1330363) found altered frequencies between cases and controls in Australian and South African populations—but did not reach statistical significance [75] (Table 5). However, the study did implicate a genetic region spanning both the TNC and the COL27A1 genes using haplotype analysis. More recently, the application of WES analyses assisted the identification of potential functional TNC variants implicating the TNC gene, specifically rs1061494 and the T-T haplotype (rs1061494-rs2104772) with increased risk of Achilles tendinopathy in a South African cohort [30]. This study therefore provided evidence that the risk susceptibility to Achilles’ tendinopathy is most likely within the TNC gene rather than within the COL27A1 gene locus.

Several TNC variants have been associated with rotator cuff injury [76,77]. Previously implicated TNC variants (rs1138545, rs72758637, rs7021589) have been replicated in a GWAS study using the UK biobank to be associated with an increased risk of rotator cuff injury [57,76]. Tashjian reported increased gene expression, for TNC (2.2×) in tears vs. controls [57] (Table 5).

5.4.2. COMP and Other Thrombospondins

COMP, also known as thrombospondin 5, is the most abundant tendinous glycoprotein. Thrombospondin 2 (THBS2) mediates cell-to-cell and cell-to-matrix interactions and is involved in cell-to-cell adhesion and ECM communication (Table 4). COMP (rs730079; rs28494505) and THBS2 (rs9505888; rs6422747) variants failed to show significant difference in Achilles tendon studies in Australian and South African cohorts [75]. No human genetic studies exist relating to the potential role of Thrombospondin 2 in tendon pathology, although its absence has been associated with connective tissue abnormalities in mice [76].

A GWAS study identified rs575224171 within the gene THSD7A encoding the endothelia protein thrombospondin type 1 domain, containing protein 7A, to be associated with increased risk of rotator cuff injury [57]. These authors hypothesised that gene variants may lead to poor rotator cuff angiogenesis, predisposing individuals towards increased tear risk. RNA sequencing analyses established a 2.6× decreased expression of this gene within rotator cuff tears vs. control tissues [57].

5.5. Elastin and Microfibrils

Elastin represents 1–10% of a tendon’s dry weight and is found in both the IFM and within the fascicles—especially around tenocytes. Microfibrils, such as the glycoprotein Fibrillin, are found in the ECM and become incorporated into insoluble microfibrils (Table 4). South African, Australian, and British cohorts did not find an association between the ELN rs2071307 variant and risk of developing Achilles’ tendon pathology [78,79]. However, the FBN2 rs331079 variant was associated with risk for Achilles’ tendon disease and ACL ruptures [78].
Table 5. Summary of genetic research and tendon injuries: Glycoproteins in the ECM and elastin and fibrillin.

| Type                              | Protein                      | Reference | Country                  | Variant                             | Pathology                  | Subjects | Controls | Comments                                                                 |
|-----------------------------------|------------------------------|-----------|--------------------------|-------------------------------------|----------------------------|----------|----------|--------------------------------------------------------------------------|
| Glycoproteins in the ECM          | Tenascin-C (TNC)             | [74]      | South Africa, Australia  | TNC—GT dinucleotide repeats         | Achilles’ tendinopathy and rupture | 144      | 127      | Association with the number of GT repeat polymorphism and the risk of tendinopathy and rupture |
|                                  |                              | [60]      | South Africa, Australia  | TNC rs13321, rs2104772, rs1330563   | Achilles’ tendinopathy and rupture | 179      | 339      | No significant associations found among variants                          |
|                                  |                              | [80]      | Spain                    | TNC rs2104772                       | Patellar tendon injury         | 15       | 0        | No difference between severity of injury and gene variants. No controls were included |
| Cartilage oligomeric matrix protein (COMP) |                      | [75]      | South Africa, Australia  | COMP rs730079, rs2849450, THBS2 rs953888 | Achilles’ tendinopathy and rupture | 178      | 340      | No significant associations found among variants                          |
| Elastin and Fibrillin            | Elastin (ELN) and Fibrillin (FBN) | [78]      | South Africa, Australia  | ELN rs2071307, FBN2 rs331079        | Achilles’ tendinopathy and rupture | 135      | 239      | No association with the ELN variant and tendon pathology Significant association between the FBN2 gene variant and tendon disease |
|                                  |                              | [81]      | UK                       | ELN rs2071307                      | Achilles’ tendinopathy and rupture | 108      | 131      | No association with the ELN variant and tendon pathology                  |
|                                  |                              | [80]      | Spain                    | ELN rs2289630                      | Patellar tendon injury         | 15       | 0        | No difference between severity of injury and gene variants. No controls were included |

Green indicates an association and red indicates no association found.

5.6. Tendon Development, Homeostasis, and Remodelling

Tendon ECM homeostasis and remodelling are maintained by complex enzyme systems. These include matrix metalloproteases (MMPs), ADAMTSs (a disintegrin and metalloproteinase with thrombospondin motifs) and ADAMs (a disintegrin and metalloproteinase), tissue inhibitors of MMPs (TIMPs), and growth factors like the transforming growth factor-β (TGF-β) families [82] (Table 6). Genetic research undertaken in this area of tendon development, homeostasis, and remodelling is summarised in Table 7.

5.6.1. MMPs, TIMPs, ADAMTSs, and ADAMs

The balance between MMPs and TIMPs is necessary to maintain tendon homeostasis and remodelling [82]. If intrinsic control of these systems is compromised by extraneous factors, the tendon’s ability to respond appropriately to loading will be affected, risking tendon disease (Table 6). No association was reported of ADAMTS2, ADAMTS5, ADAMTS14, and ADAM12 variants with Achilles’ tendon pathology in South African and Australian cohorts [83]. However, Raleigh found significant associations for MMP3 variants and AT (but not rupture) in a South African population [84]. In a British population, El Khoury found no associations in Achilles’ pathology groups overall—although subgroups did show some correlations [83]. Furthermore, El Khoury found a significant association with a variant within the TIMP2 gene rs479532 for Achilles’ tendon pathology in both a South African and Australian cohort. Different genotypes were overrepresented in the subject groups in each population [83]. Gibbon explored the MMP3 locus in an Australian cohort with AT and identified a 6A-G-C-G haplotype (rs3025058, rs679620, rs591058, rs650108) with reduced risk [85].
Table 6. Tendon development, homeostasis and remodelling.

| Families | Functions | Key Regulators | Associated Diseases |
|----------|-----------|----------------|---------------------|
| Matrix metalloproteases (MMPs) (23 family members) | Four subgroups:  
  • Collagenases  
  • Gelatinases  
  • Stromelysins  
  • Membrane-type  
  • Broad proteolytic activity against collagen and other ECM compounds | MMP3: degrades different collagen types, proteoglycans, and fibronectin, and laminin | Fluoroquinolone antibiotics, which are associated with increased Achilles rupture risk [86], can affect MMP expression in human tendons [9,87] |
| A disintegrin and metalloproteinase with thrombospondin motifs (ADAMTSs) (20 family members) | Procollagen processing  
  • ADAMTS2 and 14: regulate conversion of procollagen to collagen | ADAMTS2 gene mutation causes EDS dermatoptosis type |
| A disintegrin and metalloproteinase (ADAMS) (19 family members) | Proteases | ADAM12: a marker of skeletal muscle regeneration and binds insulin growth factor binding protein-3 (IGFBP-3) |
| Tissue inhibitors of metalloproteases (TIMPs) | Inhibit MMPs activities  
  • Balance between MMPs and TIMPs is necessary to maintain tendon homeostasis and remodelling | TGF-ß1 and growth differentiation factor 5 (GDF-5), can increase Achilles tendon strength in animals [88].  
  Mechanical loading releases TGFß-1 and aids cell growth, proliferation, differentiation, and migration, as well as cell death (apoptosis). Involved in collagen and proteoglycan synthesis.  
  GDF-5 (also called CDMP-1 (cartilage-derived morphogenetic protein 1) or BMP-14 (bone morphogenic protein 14). Expressed in developing CNS and maintenance, development and repair of bones, cartilage, and various other musculoskeletal soft tissues, including tendons. | GDF-5 gene mutations (CDMP1) implicated in Hunter–Thompson type dwarfism and in Grebe Syndrome (characterized by short stature, extra digits, and short and deformed extremities) [89–92]. |
| Transforming growth factor beta (TGF-ß) superfamily | ECM homeostasis and remodelling |  |  |
| Bone morphogenetic proteins (BMPs) (20 family members) | Grouped into subfamilies of the TGF-ß superfamily  
  Growth factors and cytokines in many tissues in the body |  |  |
| Fibroblast growth factors (FGFs) (22 family members) | Influence cell development and maturation by binding to receptors (FGFR) triggering intracellular events |  |  |
5.6.2. Transforming Growth Factor-ß (TGF-ß) Superfamily

The TGF-ß superfamily has a similar role as the MMP/TIMP system in ECM homeostasis and remodelling (Table 6). Mechanotransduction is the process of converting mechanical forces into a cellular response. Tendon exposure to increased loading (within safe physiological limits) causes tenocytes to increase collagen synthesis and enhance tendon load resistance. It is the end goal of sensible incremental training [92]. The reverse occurs with inactivity. If a tendon’s tensile load is temporarily decreased, there is a reduction in secreted ECM structures including Type I collagen and COMP [93]. TGF-ß appears to be a major regulator of tendon development secondary to mechanical loading [94]. The exact mechanism of how loading activates the TGF-ß signalling pathway appears to involve an induction of scleraxis (Scx) and other markers, such as tenomodulin [95]. This promotes the synthesis and secretion into the ECM of collagen and other ECM components. TGF-ß and GDF-5 genes functional variants have been studied with an association established for GDF-5 rs143383, but not TGF-ß rs1800469, with risk of Achilles’ tendinopathy [96].

5.6.3. Bone Morphogenic Glycoproteins (BMP)

BMPs are grouped into subfamilies of the TGF-ß superfamily and function as growth factors or cytokines. Originally studied for their effect upon bone and cartilage formation, they are recognised to have a widespread function as signallers and regulators of many organs’ development [35]. A significant association was reported in a Brazilian mixed-injury cohort for the BMP4 variant (rs2761884) in tendinopathies [97].

5.6.4. Fibroblast Growth Factors (FGFs)

FGFs are required for normal development and cell maturation. They bind to receptors (FGFR), triggering intracellular events. Salles studied a group of Brazilian volleyball players with variously located tendinopathies. However, none of the investigated FGF3, FGF10, and FGFR1 variants were associated with altered risk for tendinopathy [97]. Similarly, no associations of the same genes with rotator cuff tears in American patients were reported [98]. Conversely, Motta found significant associations of the FGF3, FGF10, and FGFR1 variants with rotator cuff disease in large Brazilian case-control study [99].

Table 7. Summary of genetic research and tendon injuries: Development, homeostasis and remodelling.

| Families                                      | Reference | Country            | Variant                      | Pathology                  | Subjects | Controls | Comments                                                                 |
|-----------------------------------------------|-----------|--------------------|------------------------------|----------------------------|----------|----------|---------------------------------------------------------------------------|
| A disintegrin and metalloproteinase with      | [83]      | South Africa,     | ADAMTS2 rs1054480           | Achilles’ tendinopathy     | 173      | 248      | No significant association with Achilles’ disease                         |
| thrombospondin motifs (ADAMTSs)              |           | Australia          | ADAMTS5 rs226794            | napathy and rupture        |          |          | ADAMTS14 associated with a later onset of disease                         |
| A disintegrin and metalloproteinase (ADAMs)  |           |                    | ADAMTS14 rs4747096          |                             |          |          |                                                                           |
|                                              |           |                    | ADAM12 rs4747096            |                             |          |          |                                                                           |
| Matrix metalloproteases (MMPs) and           | [84]      | South African      | MMP3 rs591058, rs650108, rs591058 | Achilles’ tendinopathy     | 114      | 98       | Variants significantly associated with chronic tendinopathy                |
| Tissue inhibitors of metalloproteases (TIMPs) |           |                    |                              | napathy and rupture        |          |          | No increased risk of rupture with variants                               |
|                                              | [81]      | UK                 | MMP3 rs679620 TIMP2 rs4789932 | Achilles’ tendinopathy     | 118      | 131      | Lowest risk of tendinopathy associated with combination of a MMP3 rs679620 and COL5A1 rs1272 variants |
|                                              | [83]      | South Africa,     | TIMP2 rs4789932             | Achilles’ tendinopathy     | 173      | 248      | MMP3 variant significantly associated with tendinopathy and rupture groups in males but not females or overall. TIMP2 variants overrepresented in tendinopathy group |
|                                              |           | Australia          |                              | napathy and rupture        |          |          | SNP significantly overrepresented in tendinopathy group                   |
|                                              | [85]      | Australia          | MMP3 rs679620, rs591058, rs650108, rs3025058 | Achilles’ tendinopathy | 79      | 195      | An association was found with a 6A-G-C-G haplotype (rs3025058, rs679620, rs591058, rs650108) with reduced risk for Achilles tendinopathy |
Table 7. Cont.

| Families | Reference | Country | Variant | Pathology | Subjects | Controls | Comments |
|----------|-----------|---------|---------|-----------|----------|----------|----------|
| Transforming Growth Factor-β Superfamily (TGFβ) Growth/differentiation factors (GDF) | [96] | South Africa, Australia | TGFBI rs1800469, GDF5 rs143383 | Achilles’ tendinopathy and rupture | 171 | 238 | No associations with TGFBI genotype variant GDF5 variant associated with Achilles’ disease. |
| Bone morphogenetic proteins (BMPs) | [97] | Brazil | BMP4 rs2761884 | Mixed—Achilles; Patellar; Rotator cuff; Hip abductors | 52 | 86 | Significant association with BMP4 variant for tendinopathy |
| Fibroblast Growth Factors | [97] | Brazil | FGF3 rs7932320, rs1893047, rs12574452, rs4631909, rs4980700 FGF10 rs1448037 rs900379, rs1011814, rs593307 FGFR1 rs13317 | Mixed—Achilles; Patellar; Rotator cuff; Hip abductors | 52 | 86 | No significant associations with tendinopathy |
| | [98] | USA | FGF3 FGF10 FGFR1 | Rotator Cuff tears | 175 | 2595 | No significant associations |
| | [99] | Brazil | FGF3 rs12574452, FGF10 rs11750845, rs1011814 FGFR1 rs13317 | Rotator Cuff tears | 203 | 207 | All showed significant associations with rotator cuff tears |

Green indicates an association and red indicates no association found.

5.7. Cell Death (Apoptosis) and Inflammation in Tendons

Apoptosis is a natural phenomenon in many living tissues. In tendons, damaged tenocytes’ removal is facilitated by cytokine activity. Excessive tendon loading can increase apoptosis and affects the cell population’s abilities to respond effectively to exercise with secondary effects upon the ECM leading to tendon disease [100]. The role of inflammation in chronic tendinopathy has long been debated [101]. Early research utilising microscopic examination of tendinopathic tissue and biochemical analysis reported no evidence of the normal elements associated with ‘classical’ inflammation in chronic tendon injuries [102–105]. Animal work indicated that early tendinosis was associated with tenocyte stimulation rather than apoptosis and modulated by growth factors such as insulin-like growth factor 1 (IGF-1) [106]. However, the authors were unable to comment on the chronic effect of prolonged loading on cell survival. (Table 8).
Table 8. Tendon disease: Inflammatory cascade, apoptosis, and other elements.

| Type                        | Elements or Activity | Functions                                      | Key Elements                                                                 |
|-----------------------------|----------------------|------------------------------------------------|-------------------------------------------------------------------------------|
| Inflammatory Cascade and Apoptosis | Interleukins (ILs)   | • Intimately involved in the inflammatory pathway | • Interleukin-1β (IL1B) influences cellular death, proliferation, and differentiation  
|                             |                      |                                                | • Interleukin-1 receptor antagonist (ILRN) inhibits activities of both IL1B and interleukin-1α (IL1A)  
|                             |                      |                                                | • Interleukin-6 (IL6) is found in both acute and chronic inflammation  |
|                             | Caspases (CASP)      | • Family of protease enzymes intimately involved in apoptosis | • CASP8 is a pro-enzyme central to cell death regulation                                 |
| Nitric Oxide Synthases (NOS) |                      | • Nitric oxide (NO), produced by NOS enzymes, is a free radical and important cellular signalling messenger with many functions including increased production of IL6 and IL8 [107] | • Three NOS genes, NOS1, NOS2, and NOS3, encode for nNOS, iNOS and eNOS enzymes, respectively  
|                             |                      |                                                | • These genes are designed to code for differing elements of the inflammatory cascade and control over cell death |
|                             |                      |                                                | • Gene variations associated with alterations in susceptibility to tendinopathy [108]  
|                             |                      |                                                | • NOS2 expression elevated 23-fold higher than controls within days of Achilles’ tendon injury  
|                             |                      |                                                | • NO catalysed by the iNOS isoform of NOS induces apoptosis in inflammatory cells to eradicate cells from damaged area, preventing chronic inflammation and allowing remodelling to occur [109]  |
| Angiogenesis                |                      | • Marked increase in new blood vessels in chronic tendon disease [110] | • Vascular endothelial growth factor A (VEGFA)  
|                             |                      |                                                | • Kinase domain receptor (KDR)  
|                             |                      |                                                | • Hypoxia inducible factor 1 subunit alpha (HIF1A)  
|                             |                      |                                                | • Encoded by VEGFA, KDR, and HIF1A genes, respectively [111–113]  |
| Others                      | Estrogen-related receptor beta (ERR-β) | • Function unknown  
|                             |                      | • May influence the expression of PPARG1 and ESRR-inducing regulator muscle 1 (PERM1) in skeletal muscle |                                                |
|                             | Defensins            | • Microbicidal and cytotoxic peptides made by neutrophils |                                                |
5.7.1. Interleukins

Inflammatory pathways throughout the body involve numerous elements, interacting in a complex manner and resulting in gene expression alterations, apoptosis, and detrimental changes to the ECM. The protein family of interleukins are intimately involved in the inflammatory pathway. Interleukins are upregulated in early tendinopathy and involved in the inflammatory cascade and remodelling activities [114]. September reported interleukin gene-gene interactions with \( \text{COL5A1} \) rs127772, suggesting that type V collagen may be regulated by certain inflammatory mediator proteins in the IL-1\( \beta \)-signalling pathway [115]. Altering the amount of type V collagen expression could impact \( \alpha_1(\text{V}) \) collagen chains and, thereby, the collagen tendon fibril diameter and ultimately tendon capacity (Table 9).

5.7.2. Caspases

Caspases are a family of protease enzymes that are integral to programmed cell death (apoptosis). The South African research group reported that two \( \text{CASP8} \) genotypes had significant associations with AT [116] (Table 9).

5.7.3. Nitric Oxide Synthase (NOS) Enzymes

Nell found no association with gene variants for \( \text{NOS2} \) and \( \text{NOS3} \) and AT [115]. However, Brookes reported a reduced risk for AT with the \( \text{NOS2} \) rs2779249 heterozygote variant, but no association with \( \text{NOS2} \) rs2248814 [117] (Table 9).

5.8. Angiogenesis

Angiogenesis is the formation of new blood vessels from the existing vasculature. Tendons and ligaments have a poor blood supply and low metabolic rate. Consequently, their healing capacity is low [118]. Histopathological examination of chronic AT specimens shows marked increases in angiogenesis [110]. It is hypothesized that this is triggered by mechanical loading and designed to promote tendon remodelling. Further studies have identified increased levels of pro-angiogenic expression profiles and, specifically, vascular endothelial growth factor A after tenocyte mechanical loading [119–121]. Poorly regulated angiogenesis may lead to distortion of the neat parallel collagen fibril array in the tendon ECM. Increased levels of angiogenic associated proteins have been noted in both ruptured tendons and ligaments and including degenerative tendons [121–123]. Angiogenesis elements are placed centrally within the network of partners regulating key ECM components within tendon and ligament.

Several functional variants within the \( \text{VEGFA} \) (rs699947, rs1570360, and rs2010963) gene have been explored and a risk haplotype was implicated both in a (i) South African cohort and a (ii) combined South African and British cohort of mid portion chronic AT [124]. Specifically, the \( \text{VEGFA} \) A-G-G (rs699947 C/A–rs1570360 G/A–rs2010963 G/C) inferred haplotype was associated with increased risk of AT. This haplotype includes the collective alleles associated with decreased \( \text{VEGFA} \) gene transcription and a corresponding lower \( \text{VEGFA} \) plasma level [125]. Therefore, it is reasonable to hypothesise that these allele combinations would potentially contribute to limiting the capacity of the structure to regulate ECM remodelling within a hypovascular tendon [122]. The authors did not report an association with Achilles’ tendon risk for any of the variants explored in \( \text{KDR} \) (rs2071559 and rs1870377) [123] (Table 8).

Like other gene loci, differences in associations at the \( \text{VEGFA} \) and \( \text{KDR} \) loci have been noted in populations of different ancestry. One study reported no associations in two \( \text{KDR} \) polymorphisms (rs1870377; rs2071559) with AT in South African and UK cohorts [124] (Table 9).
5.9. Other Areas of Genetic Study in Tendons

5.9.1. ESRRB

Estrogen-related receptor beta (ERR-β) is a nuclear receptor encoded by ESRRB (Estrogen Related Receptor Beta) gene. Its function is unknown; however, a similar protein in mice plays an essential role in placental development. It appears to influence the expression of PPARGC1 and ESRR-inducing regulator muscle 1 (PERM1) in skeletal muscle. Motta identified two SNPs in the ESRRB gene that were associated with rotator cuff disease [99]. Teerlink found a significant association for rotator cuff injury with an ESRRP rs17583842 variant [98] (Table 9).

5.9.2. Defensin ß1

Defensins form a family of microbicidal and cytotoxic peptides made by neutrophils. Defensin ß1 is encoded by the DFNB gene. It resists microbial organisms from attaching to epithelial surfaces. Motta found the DEFBI rs1800972 SNP to be associated with a preventive effect for rotator cuff tears [99] while Teerlink found no association with DEFB-1 [98] (Table 9).

Table 9. Summary of genetic research and tendon injuries: Inflammatory cascade, apoptosis, and others.

| Type                          | Protein       | Reference  | Country          | Variant                      | Pathology                          | Subjects | Controls | Comments                                      |
|-------------------------------|---------------|------------|------------------|------------------------------|------------------------------------|---------|----------|------------------------------------------------|
| Inflammatory Cascade and Apoptosis | Interleukins (ILs) | [116]      | South Africa, Australia | IL1B rs1143627, rs16944, IL1RN rs2234663, IL6 rs1800795 | Achilles tendinopathy and rupture | 175     | 369      | No independent associations. In combination with COL5A1 rs12722 these alleles had significant association with Achilles’ tendinopathy |
| Caspases (CASP)               | CASP8 rs3834129, rs1045485 | [115]      | South Africa, Australia | Achilles tendinopathy and rupture | 166     | 358      | Significant association with both polymorphisms |
| Nitric Oxide Synthases (NOS)  | iNOS rs2779249, iNOS rs248814, | [117]      | UK                | Achilles tendinopathy and rupture | 132     | 145      | Significant association (protective effect) with rs2779249 No association with rs2248814 |
| Angiogenesis Factors         | VEGFA rs69947, rs1570360, rs2010963, | [124]      | South Africa and UK | Achilles tendinopathy | 120     | 108      | Significant association with all 3 VEGFA polymorphisms No association with the KDR polymorphisms |
| Others                        | DEFBI         | [98]       | USA               | Rotator Cuff tears | 175     | 2595     | No association                                      |
|                               | DEFB-1 rs1800972 | [99]       | Brazil            | Rotator Cuff tears | 203     | 207      | Significant association (protective effect) |

Green indicates an association and red indicates no association found.

5.10. Ageing in Tendons

Diseased and damaged tendons increase with age. For instance, 11–37% develop tears in an ankle peroneal tendon during their lifetime [126]. Long exposure to mechanical stresses and the increasing inefficiency of tendon repair are implicated. It is a classic example of the Injury Causation model (Figure 1). As a result, performance and function are impaired [127]. Many age-related changes occur to tendons during their lifetime and differences are apparent in the way men and women respond to tendon loading [128]. Additional knowledge has been derived from animal research, especially equine studies and it has been proposed that the ability of pluripotent mesenchymal stem cells to differentiate into tenocytes reduces with time. Tendon stem cells become less numerous with age...
and their ability to differentiate and produce competent mature tenocytes reduces [129].
The senescence-inhibited gene within the tenocyte is downregulated [130]. The ageing
tenocyte’s complement of produced proteins (proteome) is restricted [131]. The proteins
affected include those with roles involved in cell survival and death, cytoskeletal changes,
and antioxidant response.

The ability to repair damaged tendons is also affected by non-collagenous ECM protein
turnover, including cytokines and various growth factors, and a disruption to the fine home-
ostatic mechanisms outlined in Section 5.6 [132]. Equine research has revealed that with age,
the tendons’ protein turnover diminishes [133], glycosaminoglycans increase [134], the type
III collagen increases proportionately [38], and collagen fibril diameter diminishes [135].
Changes occur to the IFM with reduced protein turnover and elasticity, increasing the
risk of injury [136,137]. MMP activity reduces tendon strength [127]. There is additional
evidence that age carries with it a reduced capacity to resolve inflammation [138]. In addition,
degenerative human tendons can have altered responses to reactive oxygen species
with age, and therefore oxidative stress may be an important pathway in tendinopathy
development [139].

The progressive alteration in tendon function involves a complex interplay between
many influences. This includes not only our inherited genetic material but temporal
changes to gene expression regulation, including an array of epigenetic mechanisms [140].
The potential role of epigenetics will be elaborated upon in Section 9.

6. Ligament: Structure, Function, and Genetic Research

Ligaments span joints attaching at either end to bones. Like tendons, they are fibrous,
dense connective tissues. They are designed to resist excessive load, control joint motion,
prevent instability, and have a vital proprioceptive role on account of their rich innervation.
They have a similar hierarchical structure to tendons. However, the degree of packing of
the collagen is slightly different. Whereas tendons organise collagen fibres in an orderly,
parallel orientation, ligament organisation is more random and less parallel. This allows
ligaments to respond to tensile loads in different directions. The cellular elements tend
to be more randomly distributed and rounder in shape than tendons. There is a higher
percentage of proteoglycans and water and reduced percentage of collagen. The elastin
content in ligament is higher than tendons.

6.1. Collagen

6.1.1. Type I Collagen

There has been considerable interest in the genetic architecture of the COLIA gene
and susceptibility to tendon and ligament injuries. Differences in the genetic susceptibility
to acute and chronic injuries have been linked to rs1107946 (−1997 G/T) and rs1800012
(+1245 G/T), within the COLIA1 gene. The rare TT genotype of the Sp1 binding site variant
(rs1800012) was associated with decreased risk for acute injuries such as shoulder disloca-
tions [141], ACL ruptures [47], and acute soft tissue ruptures [49]. A meta-analysis reported
the association of the rs1800012 TT genotype with reduced risk for sports-related tendon
and ligament injuries [142]. The same genotype has been associated with increased risk for
intervertebral disc degeneration in the elderly and increased risk for lumbar disc disease in
young military recruits [143,144]. The alternate rs1800012 GG genotype was reported to
reduce risk for ACL ruptures sustained while skiing [145]. The rs1107946 variant, which is
in linkage disequilibrium with rs1800012, has been independently associated with the risk
of skiing-associated ACL ruptures [146]. Haplotype analyses with these two functional
variants have been associated with ACL rupture risk in a Polish cohort [147]. The cur-
rent theory proposes that these functional promotor variants work in concert to regulate
COLIA1 expression [148].

However, several studies have failed to reflect an association between these COLIA1
variants and susceptibility to several musculoskeletal soft tissue injury phenotypes. This
may result from insufficient power of the studies to detect the rare rs1800012 TT genotype
in and may explain conflicting results when comparing data from larger combined analyses to that of smaller independent cohorts [50]. No associations were noted in the Chinese Yunnan Han ACL samples for the COL1A1 or COL5A1 locus [149]. However, the TT genotype of the COL1A1 Sp1 binding site polymorphism has been reported to be significantly underrepresented in South African participants with ACL ruptures [150] (Table 10).

Table 10. Summary of genetic research and ligament injuries: Collagen types I and III.

| Collagen Types | Reference | Country | Variant | Pathology | Subjects | Controls | Comments |
|---------------|-----------|---------|---------|-----------|----------|----------|----------|
| Type I        | [150]     | South Africa | COL1A1 rs1800012 | ACL rupture | 117 | 130 | Significant association |
|               | [147]     | Poland | COL1A1 rs1107946, rs1800012 | ACL rupture | 91 | 143 | Significant association |
|               | [146]     | Poland | COL1A1 rs1107946, rs1800012 | ACL rupture | 138 | 183 | Significant association |
|               | [142]     | Meta analyses | COL1A1 rs1800012 | Tendon and ligament injuries | 933 | 1381 | Significant association |
|               | [149]     | Chinese Yunnan Han | COL1A1 rs1800012 | ACL rupture | 101 | 110 | No significant association |
|               | [50]      | Combined population from SA, Sweden, Poland, Finland | COL1A1 rs1800012 | ACL rupture and Cruciate ligament rupture | 1425 | 407 | Significant association |
| Type III      | [151]     | Poland | COL3A1 rs1800255 | ACL rupture | 138 | 183 | Significant association |
|               | [152]     | South Africa, Poland | COL3A1 rs1800255 | ACL rupture | 333 | 378 | Significant association |

Green indicates an association and red indicates no association found.

6.1.2. Type V Collagen

As in AT research, variants within the 3’-UTR of the COL5A1 gene have been associated with ACL rupture susceptibility, specifically in females [152–154], and more recently with ligament injuries [155]. Laguette explored the intron 4-exon 5 region of COL5A1, which was previously implicated with ligament injuries in a canine model but found no significant associations in a South African ACL cohort [156]. The COL5A1 rs12722 C/T and COL5A1 rs13945 C/T polymorphisms were also associated with reduced ACL injury risk in male skiers [157]. Furthermore, Suijkerbuijk reported an association with ACL ruptures and COL5A1 rs12722 in a combined Swedish and South African cohort [158] (Table 11).
Table 11. Summary of genetic research and ligament injuries: Collagen types V and XII.

| Collagen Types | Reference | Country | Variant | Pathology | Subjects | Controls | Comments                           |
|---------------|-----------|---------|---------|-----------|----------|----------|------------------------------------|
| Type V        | [153]     | South Africa | COL5A1 rs12722, rs13946 | ACL rupture | 129      | 216      | Significant association            |
|               | [157]     | Poland    | COL5A1 rs12722, rs13946 | ACL rupture | 138      | 183      | Associated with reduced injury risk |
|               | [152]     | South Africa and Poland | COL5A1 rs12722 | ACL rupture | 333      | 378      | Significant gene-gene association  |
|               | [154]     | Poland    | COL5A1 rs12722, rs13946 | ACL rupture | 134      | 211      | Significant association            |
|               | [158]     | South Africa and Sweden | COL5A1 rs12722 | ACL rupture | 98       | 79       | Significant association            |
|               | [156]     | South Africa | COL5A1 rs3922912, rs4841926 | ACL rupture | 249      | 210      | No significant association         |
|               | [149]     | Chinese Yunnan Han  | COL5A1 rs12722, rs13946 | ACL rupture | 101      | 110      | No significant association         |
|               | [155]     | South Africa, Australia, Japan | COL5A1 rs12722, rs10628678 | ACL rupture and Ligament injury | 311 | 592 | Significant association |
| Type XII      | [159]     | South Africa | COL12A1 rs970547 | ACL rupture | 129      | 216      | Significant association            |
|               | [160]     | Poland    | COL12A1 rs970547 | ACL rupture | 91       | 143      | No significant association         |
|               | [152]     | South Africa and Poland | COL12A1 rs970547 | ACL rupture | 333      | 378      | Significant association            |
|               | [149]     | Chinese Yunnan Han  | COL12A1 rs970547, rs240736 | ACL rupture | 101      | 110      | Significant association |

Green indicates an association and red indicates no association found.

6.1.3. Types III and XII Collagen

Associations have been noted for variants in COL3A1 [151,152], and COL12A1 [149,159] with ACL rupture risk, while others reported no significant associations [147,161].

6.2. Regulatory and Structural Components of the Extracellular Matrix

6.2.1. Tenascin-C

Historically, there has been much interest in the associations between the TNC gene and its neighbouring genes with tendon and ligament injury susceptibility. Gibbon explored this region for ACL susceptibility. Variants within the TNC gene: rs2104772 and a TT haplotype (rs1061494 and rs2104772) were associated with ACL susceptibility using a tailored WES and bioinformatics approach [30]. However, no associations for the TNC locus were noted in a Polish cohort [162]. More functional research is required to understand the biological significance underpinning this genetic locus and tendon and ligament injury susceptibility.

6.2.2. Proteoglycans

Recent studies have investigated proteoglycans. Variants within their controlling genes have been implicated with susceptibility to ACL ruptures in independent cohorts from South Africa [163,164] and Poland [165] (Table 12).
Table 12. Summary of genetic research and ligament injuries: Glycoproteins in the ECM and proteoglycans.

| Type                  | Protein          | Reference | Country      | Variant                               | Pathology      | Subjects | Controls | Comments                      |
|-----------------------|------------------|-----------|--------------|---------------------------------------|----------------|----------|----------|--------------------------------|
| Glycoproteins in the ECM | Tenascin C (TNC) | [30]      | South Africa | TNC rs1061494, rs1138845, rs2104772, rs1061495 | ACL rupture     | 234      | 232      | Significant association        |
|                       |                  | [162]     | Poland       | TNC rs2104772, rs1330363, rs13321      | ACL rupture     | 229      | 192      | No significant association     |
| Aggrecan (ACAN)       |                  | [163]     | South Africa | ACAN rs2351491, rs1042631, rs1516797   | ACL rupture     | 227      | 234      | Significant association        |
|                       |                  | [165]     | Poland       | ACAN rs2351491                         | ACL rupture     | 143      | 229      | Significant association        |
| Biglycan (BGN)        |                  | [163]     | South Africa | BGN rs11264797, rs1126499, rs1042103   | ACL rupture     | 227      | 234      | Significant association        |
|                       |                  | [165]     | Poland       | BGN rs11264797, rs1042103             | ACL rupture     | 143      | 229      | Significant association        |
|                       |                  | [166]     | South Africa | BGN rs11264797, rs1042103             | ACL rupture     | 227      | 234      | Significant gene-gene interactions |
| Decorin (DCN)         |                  | [163]     | South Africa | DCN rs13312816, rs516115, LUM rs2268578, FMOD rs7543148 | ACL rupture | 227      | 234      | Significant association |

Green indicates an association and red indicates no association found.

6.2.3. MMPs

As in tendinopathy, the MMP locus (chr11q22) has been associated with susceptibility to ACL ruptures. Posthumus demonstrated that MMP3 rs679620 variant may interact with several other MMP loci, MMP10 rs485055, MMP1 rs1799750, and MMP12 rs2276109, to collectively contribute to ACL rupture susceptibility in a South African cohort [167]. The MMP3 rs3025058 variant, which is tagged by rs679620, was independently associated with ACL ruptures in a Thai population [168]. No associations were noted when MMP1 rs1799750, MMP10 rs486055, and MMP12 rs2276109 variants were explored with ACL rupture susceptibility in a Polish cohort [169]. The MMP genes have been investigated with several different exercise-related phenotypes and conflicting associations have been noted. This suggests that there may be specific genetic signatures which are inherited together and underpin specific exercise-related phenotypes, which still require functional unravelling (Table 13).

6.2.4. Transforming Growth Factor Superfamily

Variants in several such genes controlling the TGF superfamily have been explored with an association to ACL rupture risk. These include variants within the TGF-β receptor III (TGFBR3) and the TGF-β induced (TGFβI) genes. An independent association of TGFBR3 rs1805113 G allele with a decreased risk of ACL injury has been described. Additionally, a genetic interval between TGFBR3 rs1805113-rs1805117 was associated with ACL injury risk in a South African cohort [158].

GDF5 plays a critical role in tendon and ligament repair. Variant analyses within GDF5 gene have shown conflicting risk associations with ACL injury and larger studies are required to understand the significance of this locus with ACL injury risk [170,171].
6.3. Signalling Factors

6.3.1. Interleukins

Investigation of interleukins have shown similar findings to tendinopathy. An inferred allele combination (IL1B, IL6, IL6R, and COL5A1) was associated with ACL rupture risk [158,172,173]. Differences were noted at the alleles implicated for the IL1RN rs2234663 and IL6 rs1800795 loci. The functional consequence of these genetic loci was subsequently explored [157]. Cells treated with either hrIL-β or hrTNF-α expressed altered levels of BGN mRNA (which encodes for the biglycan PG) and COL5A1 mRNA depending on their IL1B-high risk or IL1B-low genotype profiles. Evidence suggests that the inflammatory micro-environment together with an individual’s genetic profile can modulate ECM expression of tendon and ligament components and thereby potentially impact these structures’ functional capacity.

6.3.2. Caspases

Both Rahim and Seale have reported associations between caspase functional gene variants and ACL ruptures [174,175].

Table 13. Summary of genetic research and ligament injuries: Development, homeostasis and remodelling and inflammatory cascade and apoptosis.

| Type                        | Protein                  | Reference | Country               | Variant                                      | Pathology    | Subjects | Controls | Comments                     |
|-----------------------------|--------------------------|-----------|-----------------------|----------------------------------------------|--------------|----------|----------|-------------------------------|
| Development, Homeostasis,   | TGFβ Growth Factor       | [156]     | South Africa          | TGFβ1 rs1442, TGFβ3 rs1805113, rs1805117     | ACL rupture  | 249      | 210      | Significant association       |
| and Remodelling             | Superfamily              |           |                       |                                              |              |          |          |                               |
| Matrix metalloproteases     |                          | [170]     | South Africa          | GDF5 rs1413383                                | ACL rupture  | 126      | 214      | No significant association    |
| (MMPs)                     |                          | [171]     | China                 | GDF5 rs1413383                                | ACL rupture  | 286      | 500      | Significant association       |
| Inflammatory Cascade, and   |                          | [167]     | South Africa          | MMP1 rs1799750, MMP3 rs679620, MMP10 rs486055, MMP12 rs2276109 | ACL rupture  | 129      | 216      | Significant association       |
| Apoptosis                   |                          |           |                       |                                              |              |          |          |                               |
|                             |                          | [168]     | Thailand              | MMP3 rs3025058, rs679620                      | ACL rupture  | 86       | 100      | Significant association       |
|                             |                          |           |                       |                                              |              |          |          |                               |
|                             |                          | [169]     | Poland                | MMP1 rs1799750, MMP10 rs486055, MMP12 rs2276109 | ACL rupture  | 228      | 202      | Significant association       |
|                             |                          |           |                       |                                              |              |          |          |                               |
|                             |                          | [173]     | Poland                | IL1B rs1143627, rs16944, IL6R rs2228145, IL6 rs1800795 | ACL rupture  | 229      | 194      | Significant association       |
|                             |                          |           |                       |                                              |              |          |          |                               |
|                             |                          | [158]     | South Africa and      | IL1B rs16944, IL6 rs1800795, IL6R rs2228145   | ACL rupture  | 79       | 116      | Significant association       |
|                             |                          |           | Sweden                |                                              |              |          |          |                               |
|                             |                          | [176]     | South Africa          | IL1B rs16944, IL6 rs1800795, IL6R rs2228145   | ACL rupture  | 234      | 232      | Significant association       |
| Caspases (CASP)             |                          |           |                       |                                              |              |          |          |                               |
| [172] South Africa          | CASP8 rs3834129          | ACL rupture | 234      | 232      | Significant association       |
| [175] South Africa          | CASP8 rs383412, rs1045485, rs13113 | ACL rupture | 102      | 116      | Significant association       |

Green indicates an association and red indicates no association found.
6.3.3. Angiogenesis

Independent and haplotype associations were noted for VEGFA functional variants (rs699947, rs1570360, and rs2010963) with ACL rupture susceptibility [172,174,177,178] including contrasting associations between ACL rupture and AT susceptibility. For example, the VEGFA rs699947 CC was associated with increased risk of non-contact ACL ruptures [174] but associated with a reduced risk of AT [124]. Similarly, the inferred haplotype, associated with increased VEGF production [125], was more often observed with an increased risk of ACL rupture whereas the low-VEGF producing haplotype was associated with a reduced risk of injury [174]. In contrast, the low-VEGF producing haplotype was associated with increased risk of tendinopathy [124]. Following a pathway-based approach, including DNA variants within the interleukin and the angiogenesis encoding genes, Rahim highlighted that VEGFA rs699947 CC, VEGFA rs2010963 GC, BMI, and age remain significant biological components in ACL rupture susceptibility [176].

Evidence suggests that a lower-level blood flow increase after running is associated with higher risk for developing AT in an age and sex-dependent manner [179]. Whereas, in the ACL model, overexpression of VEGFA may reduce the biomechanical strength of the tendon graft in the early stages of an ACL ligament reconstruction, whilst in the later stages of graft incorporation increased expression is essential [180].

Therefore, it does seem, that the “Goldilocks affect” is still plausible [115] suggesting that a finely tuned homeostatic feedback regulation of ECM components is required to maintain both tendon and ligament tissue integrity. Willard and Suijkerbuijk have shown functional evidence linking a genetic contribution, at key proteoglycan, interleukin, and collagen genes, to the expression of ECM components in a susceptibility model [158,164].

Further exploring the angiogenesis pathway and knowing the VEGF biological effects are mediated via its receptor kinase insert-domain receptor (KDR), Rahim showed that the inferred G-A KDR haplotype (rs2071559 A/G, rs1870377 A/T) was significantly associated with increased susceptibility to ACL ruptures [174]. It was suggested that the rs2071559 G allele alters a potential transcription factor binding site in the promoter region, thereby reducing KDR transcription [181] and the A allele of rs1870377 T/A was associated with reduced VEGF-binding efficiency [181]. Lulinska-Kuklik identified an association between VEGFA rs2010963 and ACL injuries in a Polish population, but not with VEGFA rs699947 or VEGFA rs1570360 [178]. These associations need to be repeated in larger ACL data sets. More recently, Feldmann investigated ACL injury risk in a combined cohort from Sweden, Poland, Australia, and South Africa, and further implicated the VEGFA rs201093 CC genotype and the VEGFA (rs699947 C/A, rs57036 G/A and rs2010963 G/C) A-A-G haplotype to be associated with reduced risk of an ACL injury [182]. In addition, the authors suggest that possibly variants in KDR are not associated with ACL risk susceptibility. The differences noted with KDR in the various populations may represent Type I statistical errors (Table 14).
Table 14. Summary of genetic research and tendon injuries: Angiogenesis and fibrinogens.

| Type | Protein | Reference | Country | Variant | Pathology | Subjects | Controls | Comments |
|------|---------|-----------|---------|---------|-----------|----------|---------|----------|
| Angiogenic Factors | Vascular endothelial growth factor receptor (KDR) | [174] | South Africa | KDR rs2071559, rs1870377, VEGFA rs699947, rs570360, rs2010963, NGFB rs6678788, HIF1A rs11549465 | ACL rupture | 227 | 126 | Significant association |
| | Vascular endothelial growth factor (VEGF) beta-nerve growth factor (NGF) | [177] | South Africa | KDR rs2071559, rs1870377, VEGFA rs699947, rs570360, rs2010963 | ACL rupture | 98 | 100 | Significant association |
| | Hypoxia-inducible factor (HIF) | [176] | South Africa | KDR rs2071559, rs1870377, VEGFA rs699947, rs570360, rs2010963 | ACL rupture | 234 | 232 | Significant association |
| | Fibrinogens | [149] | Chinese Yunnan Han | FGB rs1800789, rs1800790, rs1800791, rs2227389 | ACL rupture | 101 | 110 | No significant association |

Green indicates an association and red indicates no association found.

More informative population specific genetic variants are required to be tested [183–186]. Observing the differences and similarities between these genetic association findings with tendon and ligament injury susceptibility may highlight different biological mechanisms underpinning the two injury models (acute vs. chronic) or it may be indicative of the differences between ligament and tendon molecular-functional correlations. Moreover, it may also be reflective that larger data sets are required to comprehensively screen a gene of interest across populations. It remains essential that the genetic associations are explored at a functional level to assess the biological significance of these variants in both acute and chronic injuries.

7. Epigenetics

Despite the growing number of genetic loci implicated in tendon and ligament injury susceptibility, a large unknown heritability component remains unresolved [24]. The impact of epigenetic regulation of the genome is an emerging area of research in understanding and deciphering the heritability of common complex phenotypes. Epigenetics was first described in 1968 [187]. It refers to heritable changes (from parent to child) which do not depend upon DNA sequence changes. DNA within every one of our cells is identical in content and sequencing with a few exceptions. What determines an individual cell’s function is the gene expression within it. Differential expression will determine, for example, if a cell acts as a tenocyte or osteoblast.

DNA must be accessible for transcription by RNA polymerase to be active. Genetic material transcription ‘visibility’ can be controlled by several mechanisms, which are important during development and differentiation of cellular components and tissues. Equally, they are important in influencing the way that mature tendons and ligaments respond to normal adaptation to loading and pathological disease evolution.

The main epigenetic mechanisms facilitating protein expression regulation, without altering the DNA sequence, involve chemical modifications to the genome and DNA, and the associated proteins. This occurs through either DNA methylation, histone modifications and the actions of non-coding RNAs (ncRNA). The latter is an RNA molecule produced by DNA, but not translated into protein. They regulate gene expression at both the transcriptional and post-transcriptional stages of protein synthesis. This type of RNA includes...
micro-RNAs. They influence and act alongside other epigenetic mechanisms to create ‘gene silencing’. Parts of epigenetic regulation can be inherited via the germline. Others result from a response to an individual’s environmental exposure, which can include but is not limited to lifestyle choices. Epigenetic mechanisms may play an important part in explaining the variation of phenotype in complex disease processes. This includes the lack of reproducibility in some linkage and association studies, observed twin discordance, and different ages of disease-onset when subjects possess the same genotype [188].

7.1. DNA Methylation

Some of the genes/gene families implicated in either tendon or ligament injury risk susceptibility have been associated with altered methylation status [189]. For example, a hypermethylation status, typically associated with reduced gene expression, of several CpG sites within the promoter regions of the MMP11 and ADAMTS4 genes but not TIMP2 was noted in pathological human patellar tendon samples compared to control tendon tissue samples [190,191]. The MMP11 and ADAMTS4 enzymes play an important role in ECM regulation and in particular PGs such as aggrecan. Enzyme reduction could result in substrate accumulation. An increased expression profile of large proteoglycans, such as aggrecan, was reported in pathological human patellar tendons [192,193].

The methylation status, together with the corresponding mRNA expression profiles of several MMPs (MMP1, −2, −3, −9, −13, and −14) and TIMP (TIMP1, 2 and 3) genes, was investigated using ruptured shoulder supraspinatus tendon tissue [194]. Significantly altered DNA methylation patterns for MMP1, MMP9, MMP13, TIMP2, and TIMP3 were described at the torn tendon edge compared to uninjured control tendon samples. An inverse correlation was noted between the overall methylation status of promoter sites within MMP1, −9, −13, and TIMP3, including the 5’-UTR of TIMP3, and the respective mRNA expression. Changes in the methylation pattern of some of these genes were partly influenced by age at surgery, sex, smoking habit, tear size, and/or duration of symptoms, which are some of the confounders previously implicated in injury susceptibility and/or impaired tendon healing [158].

7.2. RNA Interference

There has been increased interest in miRNAs and miRNA binding sites and their role in protein expression regulation at the post-translational level. In particular, the association of the putative hsa-miR-608 binding site within the 3’UTR region of COL5A1 gene with both AT and ACL rupture susceptibility [54,58].

The miRNA-29 family, for which direct/indirect targets include collagens, MMPs, integrins and some DNA methylases have been examined [195]. No significant differences in expression profile were noted in torn supraspinatus tendons versus uninjured tendon samples. However, there was an inverse correlation between the expression profiles of hsa-miR-29a-3p, hsa-miR-29b-3p and -5p and MMP2, MMP9 and MMP14 expression. hsa-miR-29a-3p and miR-29b-5p were inversely correlated with MMP1 expression [194]. This suggests that the miRNA-29 family contributes to regulating specific ECM components of rotator cuff tendons.

Several potential epigenetic candidate loci and regulators have been highlighted in both tendon and ligament biology. However, little has been explored in the context of tendon and ligament injury and susceptibility [196].

8. Discussion

This review has highlighted both overlaps and distinctions in the genetic loci implicated between tendon and ligament injuries, as well as for acute and chronic injuries of both. This underlines the subtleties within musculoskeletal soft tissue pathology. Underlying factors behind acute, subacute, and chronic pathology are likely to be very different. For acute injuries, extrinsic factors will probably dominate—irrespective of the genotype of the individual involved, e.g., an impact injury. More chronic problems (and those who fail to
recover from acute injuries) are more likely to identify intrinsic factors, including genetic, as part of the underlying disease pathway.

It is important for researchers to avoid categorising all tendon and ligament issues as having a common pathology. Structural changes can affect different parts of the tendon and ligament. For example, the Achilles tendon can have problems arising from within both the main tendon and its calcaneal insertion. The anatomy, physiology, and biomechanics will vary according to site. Within the main tendon, changes can affect the paratenon or main tendon. The medially placed plantaris tendon can be the primary source of ‘Achilles’ pathology’. The tendon may suffer small splits and partial tears or, more commonly, tendon enlargement. An acute rupture needs to be categorised differently to a decade old swollen tendon. These different subsets of potential pathology highlight the importance of the care required when assembling cohorts for genetic research analysis.

It is becoming evident that tendon and ligament matrix remodelling is complex and, therefore, not surprising that genetic loci controlling both structural pathways and regulators of ECM homeostasis have been implicated in injury susceptibility. There appears to be a lag in progression in understanding the functional significance of most of these loci. Preliminary functional theories have been presented for \textit{COL1A1}, \textit{COL5A1}, and for the \textit{VEGFA} loci. It is critical that (i) genetic loci are explored in large data sets, (ii) explored in multiple populations, (iii) and that the functional significances of these loci are explored at the cellular and tissue levels for us to (iv) start determining the clinical relevance of these genetic contributions to normal adaptation and injury susceptibility, recovery, and tissue capacity.

One reason for a lag in expression and functional analyses, is access to pathological tissue to explore gene-phenotype correlations and impact on these tissues of time on the “remodelling curve”. Tendons and ligaments are dynamic tissues capable of responding to changing environments and the capacity to be influenced by both environmental, genetic and non-genetic factors supporting and guiding ECM remodelling. The contributions to date of genetic research on tendon and ligament injury and disease have represented no more than the identification of small pieces in a huge jigsaw puzzle. We are still to fully understand the exact position, importance, and relationships of these pieces.

Most research to date has followed a case-control genetic association approach and have been relatively restricted in location and subject and control ethnicity. The need for larger, more highly powered studies is essential. The ability to replicate the findings of the early studies in more geographical locations and representing more diverse ethnic groupings is essential. The era of omics and high throughput technologies is well-established and there is a growing trend for its application in tendon and ligament injury susceptibility. These approaches are highlighting a few of the susceptibility loci identified in the case-control studies such as \textit{TNC} and \textit{COL5A1}. However, the large majority of these have not been identified in GWAS, whole genome sequencing (WGS), and WES analyses.

Poorly defined cases and controls have been a limitation of the hypothesis-free approaches to date. Moving forward, cases and controls recruited to genetic studies should be classified as carefully as possible based on the most appropriate clinical and imaging assessment tools. Exposure to other confounding risk factors, an injury profile of the full spectrum of tendon and ligament or any other connective tissue injuries, medication history, pain symptoms, flexibility and other relative measurements need careful documentation. Failure to do so will reduce the probability of identifying important genetic factors at various stages of the underlying pathology, as well as improving our understanding of the capacity of the tissues to resist, respond, and recover from load.

Future work should attempt to determine the relative contribution of different genetic factors on overall risk and the potential influence of epigenetic factors affecting gene expression. Once we have more puzzle pieces fitting, we should be in an improved position towards a golden opportunity of improving patients’ care, towards reducing and preventing musculoskeletal conditions [197]. The ‘holy grail’ of genetic research in this field is a better understanding and management of soft-tissue pathology utilising
laboratory-based findings during clinical work. This may arise as part of a preventative programme recognising certain individuals increased susceptibility towards developing certain disorders. Equally, it might identify which patients should respond better to different treatment strategies or improve understanding of why certain management avenues have failed.

The ethical considerations of using such data in a clinical or sporting context must be considered. Within clinical professions, understanding the subtleties of such research results is more readily appreciated when interpreted in a medical context. However, the same does not necessarily apply within a sporting context—particularly in elite sport. Ideally, polygenic profiling of identified gene candidates and scientifically assigned weighting to their importance would allow athletes to be scored to determine relative risk of developing tendon or ligament injury. Preventative programmes could be instituted to minimise risk. However, at this stage, such profiling only identifies relative risk. It carries no certainty of freedom from, or the development of, certain problems. Ethical issues will occur if athletes are selected for, or more importantly denied, sporting opportunities based on such research. Already companies exist globally providing genetic profiling and major sports organisations have screened their athletes [198]. Our level of understanding as clinicians and biologists has not yet reached the point where such developments should be condoned.

9. Conclusions

The understanding of tendon and ligament structure, function and pathology and potential genetic influences has increased dramatically in the last decade. This review highlights overlaps and distinctions in the genetic loci implicated in tendon and ligament injuries. It also highlights the need for improved study designs, including well phenotyped participants, larger samples sets, increased utilisation of next generation sequencing technologies, and functional studies of implicated loci towards improved understanding of the molecular mechanisms of these genetic loci in injury biology and their clinical significance. This would facilitate steps towards improved management of the large numbers of problems presenting in health care settings.

Our present knowledge levels are still imprecise and subject to contradictions as new emerging research and technologies appear. Consequently, our ability to translate this information into meaningful patient interventions remains limited. However, the authors are confident that, with the present level of advances in research, the ability to fuse scientific work and clinical applications will emerge in the short- to medium-term.

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