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Gene Variants that Predispose to Achilles Tendon Injuries: An Update on Recent Advances

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1. Introduction

There are a number of injuries that affect the Achilles tendon and surrounding tissues. Injuries that affect the surrounding tissues include bursitis and paritendonitis, while Achilles tendinopathy and complete or partial ruptures affect the tendon tissue itself (Puddu, 1976). Although injuries to the Achilles tendon are common as a result of participation in physical activities, Achilles tendon injuries can also occur in sedentary individuals (Young et al., 2005). Although the biological and molecular mechanisms responsible for Achilles tendon injuries are largely unknown, both intrinsic and extrinsic risk factors have nevertheless been implicated in the aetiology of these conditions (Figure 1) (Meeuwisse, 1994; Riley, 2004; September et al., 2006). Genetic susceptibility, which will be the focus of this review, has more recently been included as one of the intrinsic risk factors for chronic Achilles tendinopathy. As illustrated in figure 1, many of the intrinsic risk factors associated with Achilles tendinopathy are in their own right complex phenotypes determined by both genetic and environmental factors. Flexibility (Battie et al., 2008), biological age (Newman et al., 2010), muscle strength (Stewart et al., 2006), weight (Herrera et al., 2010) are all determined by genetic and environmental factors, the development of the male sex on the other hand is determined genetically (Kousta et al., 2010).

A brief summary of the macromolecular structure of tendons is required to understand and review our current knowledge of the genetic basis of Achilles tendon injuries. Tendons have a highly ordered hierarchical structure made up of tightly packed bundles of fibrils consisting predominately of type I collagen fibres (60% of the dry mass of tendons) (Silver et al., 2003). Other quantitively minor collagens, such as type III, V, XIV and XVI collagens form heterotypic fibrils with type I collagen or are associated with the surface of the fibrils

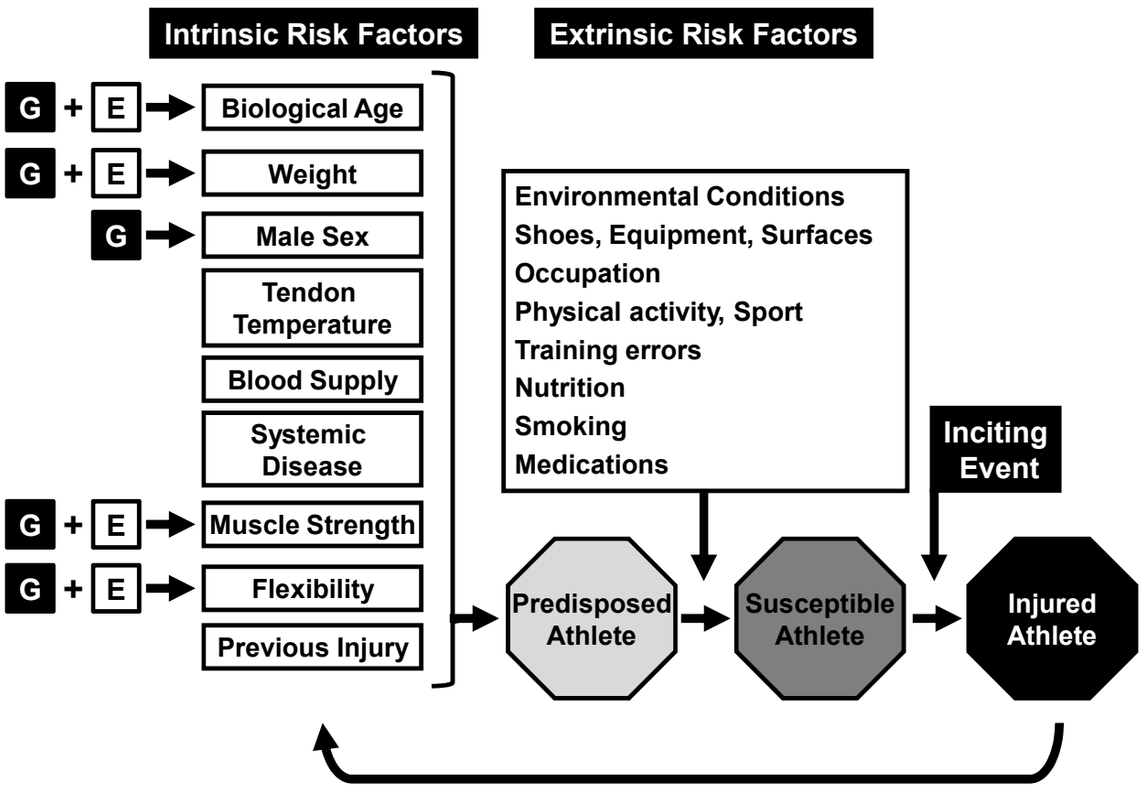

Fig. 1. A diagram illustrating the role of intrinsic and extrinsic risk factors, as well as, the inciting event in the aetiology of Achilles tendon injuries (Meeuwisse, 1994; Riley, 2004; September et al., 2006). Many of the individual intrinsic risk factors are multifactorial phenotypes, which are determined by, to a lesser or greater extent, both genetic (nature, G) and environmental (nurture, E) factors. For acute Achilles tendon injuries the inciting event will be the macrotraumatic event that cause the injury, while the inciting event for a chronic injury will be the point in time when the volume of accumulated micotraumatic damage to the tissue becomes symptomatic. The arrow from the injured Athlete back to list of intrinsic risk factors indicated that once recovered the previous injury predisposes the athlete for additional injuries.

(Canty & Kadler, 2002). These additional collagens play essential roles in tendon biology. Various glycoproteins and proteoglycans, such as tenascin C, decorin, biglycan, aggrecan, lumican, fibromodulin and others, are also important structural components of tendons (Kannus, 2000; Silver, 2003). The expression of many of these protein components has been shown to be altered during tendon injury (Ireland et al., 2001; Alfredson et al., 2003). Many collagen types, cell adhesion molecules, proteoglycans and, matrix metalloproteainases (MMPs), ADAMTS (A Disintegrin And Metalloproteinase with Thrombospondin Motifs - a family of peptidases), tissue inhibitors of metalloproteinases (TIMPS), cell receptors, cytokines and other signalling molecules are either up- or down-regulated in degenerative Achilles tendons (Ireland et al., 2001).

2. Genetic association studies with relevance to injury of the Achilles tendon

Genetic association studies are designed to investigate whether a particular allele or genotype significantly co-segregates with a particular disease trait (Lewis, 2002). Since 2005 such studies have successfully been employed to establish the identity of risk variants for Achilles tendon injuries (Collins & Raleigh, 2009). To date, genetic association studies that are relevant to Achilles tendon injuries have been based on the case-control, candidate gene approach (Collins & Raleigh, 2009). These studies rely on the accurate ascertainment of a clinically distinct phenotype which, as discussed above, can be a challenge for pathology or injury related to the Achilles tendon. In addition to the quality of the phenotypic data the design of a genetic association study must take into account potential confounding factors such as population stratification (Lewis, 2002). For multifactorial conditions such as Achilles tendon injuries other intrinsic factors, such as, amongst others, age and sex, as well as, extrinsic factors, such as type of sporting codes and duration of exposure to high risk activity, should also be considered when selecting cases and controls. The severity of the injury should also be considered when defining the inclusion and exclusion criteria. With respect to chronic Achilles tendinopathy, the following inclusion criteria have previously be used to define a severe phenotype (i) symptoms greater than 6 months, (ii) bilateral Achilles tendinopathy, (iii) multi-injuries, (iv) other tendon injuries and/or (v) early age of initial onset of symptoms (Mokone *et al.* 2005). The selection of appropriately matched controls is as important as the selection of the cases.

For the candidate gene approach, investigators select variants that are plausible *candidates* for a role in the pathology and determine whether an allele or genotype appears at a significantly greater frequency in cases compared to a matched control group (Cordell &, Clayton, 2011). The genome wide approach (GWA) tends to use large numbers of cases and controls that are genotyped for many thousands of tagged single nucleotide polymorphisms (SNPs) (Hosking *et al*, 2011). Investigators predominantly use Affeymetrix or Illumina technology for these investigations but genome wide significance is generally set within the order of $P<0.0000001$ to account for multiple testing (Grant & Hakonarson, 2008). Although GWA studies have clearly advanced our understanding of a number of complex diseases (Grant & Hakonarson, 2008) the technology has not yet been utilised in relation to gene variants that predispose to injuries of the Achilles tendon. In the following section we update our knowledge of gene variants that have been associated with Achilles tendon injuries. Our main focus of this article will be to update the reader on the most recent advances (from 2010 onwards) in the field. However, although we will begin with a brief review of some of the studies that prompted investigators to the search for specific gene variants that could influence the risk of Achilles tendon injuries (section 3) the reader is also advised to consult earlier reviews (September et al. 2006; 2007; Magra and Maffulli, 2007, Collins & Raleigh, 2009).

3. Initial investigations

In 1989 Jozsa and co-workers conducted a retrospective study on the frequency of different blood groups in a Hungarian population that had suffered from tendon ruptures (Jozsa *et al,* 1989). They found an abundance (53%) of blood group O in their cohort compared to 31% of the control sample. The abundance of group O was even higher (69%) in individuals who had sustained a re-rupture (Jozsa *et al,* 1989). Subsequent studies by Kujala and colleagues

(Kujala *et al*, 1992) and Kannus and Natri (Kannus & Natri, 1997 as cited by September, 2007) have documented associations between Achilles tendon rupture and blood group distribution. Interestingly, the relationship between blood group distribution and risk of Achilles tendon rupture was not observed in subsequent work involving 215 Achilles patients recruited in a Finnish cohort (Leppilahti *et al*, 1996), 78 patients in a Scottish based cohort (Maffulli *et al*, 2000) and in a small South African based study involving 75 rupture cases and 131 controls (Mokone, 2006 as cited by September 2007).

Despite the contrasting findings, Mokone and colleagues (Mokone *et al*, 2005) speculated that variants residing in genes encoding tendon structural or regulatory proteins, that were proximal to the ABO chromosome locus (on 9q34) might be candidates for association with Achilles tendon injuries. With this in mind, Mokone and co-workers conducted the first case-control, genetic association study using the candidate gene approach for risk variants relating to Achilles tendon injury. Using a sample of 114 Achilles tendon sufferers and 127 matched controls they established that a dinucleotide repeat polymorphism within the tenascin-C gene (*TNC*), a gene encoding an important enzyme that regulates cell matrix interactions (Jones & Jones, 2000) was associated with Achilles tendon injuries (Mokone *et al*, 2005). Interestingly the study demonstrated that the odds ratios, a quantitative estimate of disease risk based on the carrier of an allele or genotype in cases divided by controls (Lewis et al, 2002), was found to be high. Indeed possession of the 12 and 14 repeat alleles co-segregated with a six fold increase in injury risk (Mokone *et al*, 2005).

Attention was then focused on genomic variation within the gene that encodes the pro-α1(V) chain of collagen type V (*COL5A1*) as a possible susceptibility locus for Achilles tendon injuries. Specifically, using a South African based cohort two polymorphisms were selected within the *COL5A1* gene and one of them (rs12722) was found to significantly associate with chronic Achilles tendinopathy (Mokone *et al*, 2006). The DpnII restriction fragment length polymorphism also investigated by this group was not associated with injury or tendinopathy of the Achilles tendon (Mokone *et al*, 2006). Both these polymorphic markers are located within the 3'-untranslated region (UTR) of the *COL5A1* gene (September *et al*, 2009). Since 2006 confirmation of the association of the rs12722 polymorphism in Achilles tendon pathology has been documented in an Australian population, both as a single marker, and as an inferred haplotype in combination with the C allele of the rs3196378 polymorphism (September *et al*, 2009). In addition to *COL5A1*, three variants within the *MMP3* gene were also found to associate with Achilles tendinopathy in South Africans (Raleigh *et al*, 2009). Interestingly previous (Alfredson *et al*, 2003, Ireland *et al*, 2001) and more recent data (de Mos *et al*, 2009, Jelinsky *et al*, 2011) have shown that MMP3 expression levels are significantly repressed in tissue obtained from Achilles tendinopathic material when compared to controls.

4. Contemporary investigations

Although a diverse group of candidate genes for Achilles tendon injuries were suggested in 2006 (September *et al*, 2006) association studies up to and including 2010 have been limited to variants within genes for proteins involved in the structural or regulatory integrity of tendon or the extracellular matrix (Like *COL5A1* and *MMP3* respectively). To broaden the spectrum of possible candidates, Posthumus and co-workers speculated that members of the transforming growth factor-β (TGF- β) family might influence

predisposition to the risk of these injuries (Posthumus *et al*, 2010). They chose this group as logical candidates based on the biochemical functions that TGF-β transcripts had in relation to Achilles tendon function. For example, it was known that TGF-β transfected into healing rabbit Achilles tendon led to enhanced mechanical strength of the tendon due to the regulation of collagen turnover and cross link formation (Hou *et al*, 2006). However, the functional promoter variant rs1800469 of the *TGF*-β gene was not found to co-segregate with Achilles tendon pathology in both South African and Australian Caucasian cohorts (Posthumus *et al*, 2010).

The same investigators then examined the possible role of another variant (rs143383) within a second member of the TGF-β family, namely the growth differentiation factor 5 (*GDF-5*) gene, for association with Achilles tendon injury. They found that carriage of the TT genotype at the rs143383 locus was associated with Achilles tendon pathology in Australian cases and when the data were combined for both cohorts of Australian and South Africans (Posthumus *et al*, 2010). At present a mechanism for how the rs143383 variant exerts its influence on risk of Achilles tendon injury is unknown. However, it is interesting to note that the T allele of this variant has been reported to repress the expression of GDF-5 in tissue obtained from osteoarthritis patients (Egli *et al*, 2009). Furthermore, genotype at this locus (possession of either the T or C allele) has also been shown to govern the binding of the deformed epidermal autoregulatory factor 1 (DEAF-1) transcription factor which may have a role in osteoarthritis susceptibility (Egli *et al*, 2009). Accordingly, genetic variants within the DEAF-1 gene might also be candidates for Achilles tendon injuries. It is also noteworthy that the rs143383 variant has been associated with a range of other musculoskeletal pathologies. For example the rs143383 variant has been shown to be a susceptibility locus for osteoarthritis of the knee (Valdes et al, 2011) and a recent large-scale study in European cohorts has documented that the T allele of rs143383 was associated with lumber disc degeneration in women (F.M Williams *et al*, 2011).

So far we have seen how population-based association studies have enhanced our understanding of the genetic risk factors that can predispose to Achilles tendon injuries. A summary of this data (including only significant associations discussed in sections 3 and 4) is shown in Table 1.

Although studies up to 2010 have focused on single genetic loci or haplotypes that can predispose to Achilles tendon injuries a recent study has utilised a pathway-based model to study predisposing genotypes for Achilles tendon pathology (September et al, 2011). Specifically, the investigators wanted to establish whether allelic variants within a selction of inflammatory genes (interleukin-1β, interleukin-6 and interleukin-1 receptor antagonist), that are differentially expressed in tendinopathy (September *et al*, In Press), were associated with the problem when combined with the *COL5A1* rs12722 variant. Interestingly, they discovered that as single loci none of the variants associated with Achilles tendinopathy. However, in combination, the five variants tested, along with the *COL5A1* variant, were significantly associated ($p<0.005$) with Achilles tendinopathy (September *et al*, 2011). This work demonstrates that polygenic profiling of a complex phenotype like Achilles tendinopathy maybe a superior strategy to use, compared to the single candidate approach, when it comes to understanding the intricate involvement of numerous variants that increase the risk of Achilles tendon problems (September et al, 2011).

Gene	Variant/population	Notes	Reference
Tenascin C (*TNC*)	GT repeat variant within intron 7. South African Caucasian (N=241).	12 and 14 GT repeat variants associated with Achilles tendon injuries. Both allelic and genotypic associations were observed.	Mokone *et al*, 2005
Type V Collagen, α 1 chain, (*COL5A1*)	rs12722 South African Caucasian (N=240).	A2 allele overrepresented in control subjects inferring a protective role for this allele.	Mokone *et al*, 2006
Type V Collagen, α 1 chain, (*COL5A1*)	rs12722 and rs3196378 Australian Caucasian (N=295).	The CC genotype of rs12722 underrepresented in Achilles tendinopathy The rs12722 and rs3196378 variant associated with tendinopathy as an inferred haplotype	September *et al*, 2009
Matrix metalloproteinase 3 (*MMP3*)	rs679620, rs591058 and rs650108 South African Caucasian (N=212).	The GG genotype of rs679620 associated with Achilles tendinopathy. The rs679620, rs591058 and rs650108 variants also associated as an inferred haplotype	Raleigh *et al*, 2009
Growth differentiation factor 5 (GDF-5)	rs143383 Australian and Australian in combination with South African Caucasian (N=406).	The TT genotype of rs143383 overrepresented in Australian and combined Australian and South African	Posthumus *et al*, 2010

Table 1. Genetic variants associated with Achilles tendon pathology in humans. Entries summarise the results of separately published studies investigating a single variant or a haplotype. Information on individual variants can be found by databases hosted by the NCB1 available at http://www.ncbi.nlm.nih.gov/projects/SNP/.

4.1 The *COL5A1* 3'-UTR is functional and associated with other exercise-related phenotypes

Although a genetic association of a sequence variant (rs12722) within the *COL5A1* 3'-UTR has previously been reported for chronic Achilles tendinopathy (Mokone *et al*, 2006; September et al, 2009) in two independent populations, the biological function, if any, of the *COL5A1* 3'-UTR was initially unknown. The 3'-UTR of many eukaryotic protein-coding genes contains regulatory elements, such as miRNA binding sites, involved in the etiology of many diseases (Mazumder et al., 2003). In addition variant rs12722 within the *COL5A1* 3'-UTR is in close proximity with two polymorphic putative miRNA binding sites within the *COL5A1* 3'-UTR (September *et al*, 2009). Due to the structural similarities of tendon and ligaments at the molecular level, the association of the *COL5A1* rs12722 variant with another musculoskeletal soft tissue injury, namely anterior cruciate ligament (ACL) rupture, was investigated. Within females the, CC genotype of SNP rs12722 was significantly under-represented among the cases (Posthumus *et al*, 2009). Although it well document that females are at greater risk for ACL ruptures, the reason for this sex-specific association remains unknown.

Both an increase and decrease in joint range of motion (ROM) is a modifiable risk factor for Achilles tendon injuries (Brown et al., 2011a). In addition, *COL5A1* haploinsufficiency is a common molecular mechanism causing the classic form of Elhers Danlos Syndrome (EDS), which presents with amongst other symptoms joint hypermobility (Malfait et al., 2010). Brown *et al* (2011a) recently reported that the *COL5A1* CC genotype 'protected' individuals against an age-related decline in ROM measurements. Finally the 'less flexible' *COL5A1* TT genotype has been shown to be significantly associated with improved endurance running performance (Posthumus *et al*, 2011; Brown *et al*, 2011b). This finding is in agreement with the published inverse relationship between musculotendineous stiffness and running economy (Arampatzis *et al*, 2006; Dumke *et al*, 2010; Fletcher *et al*, 2010).

As reviewed above the *COL5A1* rs12722 SNP has been associated with Achilles tendinopathy and other exercise-related phenotypes. This single nucleotide DNA sequence variation is not necessarily the cause of these phenotypes. Genetic association studies do not prove cause and effect but highlight genetic regions, proteins or biological pathways that should be further investigated using other biological techniques. This rs12722 SNP is however probably tightly linked to the as yet unknown phenotype-causing polymorphism(s) either within the 3'-UTR of the *COL5A1* gene, other regions of *COL5A1* or a neighboring gene. The results do however indicate that SNP rs12722 is a representative genetic marker for the genetic region (locus) within or surrounding the *COL5A1* gene, which may potentially cause these reported phenotypes.

To test whether the *COL5A1* 3'-UTR was functional, Laguette *et al* (2011) cloned the *COL5A1* 3'-UTR from participants with chronic Achilles tendinopathy or asympomatic controls upstream of a firefly-luciferase reporter gene and transiently transfected the clones into HT1080 cells. They reported an overall increase in COL5A1 mRNA stability in the tendinopathic phenotype and identified two major functional forms of the *COL5A1* 3'-UTR. The one functional form corresponded to the wild type sequence, includes the C allele of SNP rs12722, and was identified in most of the clones generated from asymptomatic controls. The second functional form, on the other hand, included the T allele of SNP rs12722 and was predominantly identified in the Achilles tendinopathic patients. An overall

increase in mRNA stability was asscoiated with the second functional form of the COL5A1 3′-UTR, which was cloned from participants with chronic Achilles tendinopathy (Laguette *et al*, 2011).

The *COL5A1* gene encodes the α1 chain of type V. Although present in much smaller amounts than type I collagen, type V collagen plays a critical role in the regulation of type I collagen fibril assembly and lateral growth (fibrillogenesis) (Wenstrup *et al*, 2011). There is an inverse relationship between fibril diameter and type V collagen content, increased type V collagen content in the fibril causes thinner fibres. This in turn is believed to alter the mechanical properties of tissues such as the Achilles tendon (Collins & Posthumus, In Press). Type V collagen is therefore an important structural component of tendons and other connective tissues. In addition, since both copies of the *COL5A1* gene are required for normal collagen fibril formation (Wenstrup *et al*, 2006; Malfait *et al*, 2010), it is possible that relatively small changes in *COL5A1* mRNA stability within the normal physiological range (non-pathological) could result in inter-individual variation in fibrillogenesis, mechanical properties and susceptibility to musculoskeletal soft tissue injuries, as well as, variations in flexibility and endurance running performance (Collins & Posthumus, In Press).

4.2 Fibrilogenesis and Achilles injuries

As described above a variant within the functional 3′-UTR of *COL5A1* is associated with chronic Achilles tendinopathy, another musculoskeletal soft tissue injury and other exercise related phenotypes. In addition we have mentioned that type V collagen is essential for life and is an important protein regulating fibrillogenesis in tendons and other connective tissues. Other proteins besides type V collagen also regulate fibrillogenesis and therefore the gene encoding these proteins should also be considered ideal candidate genes for chronic Achilles tendinopathy.

Other proteins involved in fibrillogenesis, including type XI, XII and XIV collagens, the proteoglycans, decorin, lumican and fibromodulin, as well as the matricellular protein, thrombospondin 2 (Fichard *et al*, 1995; Reed & Iozzo, 2002; Chakravarti, 2002; Bornstein *et al*, 2000). In addition, like tenascin C which is regulated in tendons by mechanical stress, type XII and type XIV collagens are also expressed in both tendons and ligaments and regulated by mechanical stretch (Chiquet, 1999; Nishiyama *et al*, 1994). It has also been postulated that type XII and XIV collagens play an important role in the regulation of fibril assembly due to their ability to interact with proteoglycans such as decorin, lumican and fibromodulin (Ezura *et al*, 2000; Svensson *et al*, 2000; Danielson *et al*, 1997). Immunoelectron microscopy has shown that both these collagen types are associated with the surface of collagen fibrils, suggesting that they might possible be able to form interfibrillar connections and mediate fibril interaction with other extracellular and cell surface molecules (Schuppan *et al*, 1990; Keene *et al* 1991; Zhang *et al*, 1993; Walchli *et al*, 1994). Both type XII and XIV collagens are homotrimers and belong to the sub-family of fibril-associated collagens with interrupted triple helices (FACIT) and are encoded for by the *COL12A1* and *COL14A1* genes, respectively (Shaw & Olsen, 1991; Mayne & Brewton, 1993; Olsen, 1995). Variants within *COL12A1* and *COL14A1* were however not associated with these injuries (September *et al*, 2008). Although some of the tested variants were non-synonymous (changed an amino acid in the protein), we cannot exclude the possibility that other untested variants within

COL12A1 and *COL14A1* are associated with Achilles tendinopathy. Interestingly the variants within the *COL12A1* gene were however associated with ACL ruptures in females (Posthumus *et al*, 2010). The association of other genes encoding for proteins involved in fibrillogenesis remains to be tested.

5. Future research, applied and clinical significance

Several genetic markers located within genes encoding for tendon structural proteins, extracellular proteinases and signaling molecules have been shown to be associated with chronic Achilles tendinopathy. These results indicate that the genetic contribution for these injuries is polygenic and that several biological pathways are involved. The polygenic natures of Achilles tendon injuries is not surprising, since, as illustrated in figure 1, many of the intrinsic risk factors are determined by both genetic and environmental factors.

Most of the reported associations have been confirmed in a second population (September et al, 2009; Posthumus *et al*, 2010). The sample sizes of the study population have however generally been small and therefore such studies should be repeated in other populations as well as non-Caucasian populations. The association of these variants should also be tested in Achilles tendon ruptures. Interestingly, the preliminary findings suggest that there might be similarities and differences when identifying genetic elements associated within ruptures and tendinopathy (e.g. Mokone *et al*, 2005; 2006).

Although human DNA is over 99.9% identical (Burton et al., 2011), the 0.1% sequence differences (polymorphisms) partially explains why (i) every athlete is not identical (biological variation), (ii) every athlete's Achilles tendon structure is not identical, (iii) the tendons response to loading is not identical, and (iv) their response to healing (treatment modalities) is not identical. As previously discussed in an editorial (Collins, 2010), these factors fall within the developing disciple of personalized medicine. With this in mind, genetic markers could one day be included in multifactorial models to explain inter-individual variation in susceptibility to injury, as well as, response to training, prevention programmes, treatment and rehabilitation. Much more work is however required before this becomes a reality. It is however important to reiterate that Achilles tendon and other musculoskeletal soft tissue injuries are all multifactorial in nature. There is no single factor that causes any of these injuries. The inclusion of genetic markers into any model that has clinical applications could never be used for diagnostic purposes. Their inclusion will only help in determining risk. The inclusion of genetic risk factors in risk models raises ethical issues, which need to be addressed before any clinical service becomes a reality (Collins, 2010; A.G Williams & Wackerhage, 2009).

Besides the obvious clinical applications, the identification of genetic elements associated with Achilles tendon injuries will compliment the other biological disciplines in understanding and elucidating the biological mechanisms of these injuries. This is a less discussed and appreciated application. It is however currently an important application of this area of research. Some of the previously reported associated genetic markers are functional. It is therefore possible to postulate how these genes, or more specifically their protein products are involved in the etiology of the injuries. These possible mechanism need to be proved or verified using other biological techniques. As previously reviewed (Collins

& Posthumus, In Press) work is currently ongoing to explain how variants within the 3'-UTR of the *COL5A1* gene could be directly involved in the susceptibility to Achilles tendon injuries. One of the reasons the current risk models (figure 1) have limited practical application in determining risk of injury for a individual athlete is that they are not based on an understanding on the biological mechanisms causing the injury.

We propose that future risk models should be developed around an understanding of the biological mechanisms of Achilles tendon injuries (Figure 2). Current and future research using human molecular genetics and other biological techniques will play an important part in elucidating the mechanisms and developing more appropriate risk models.

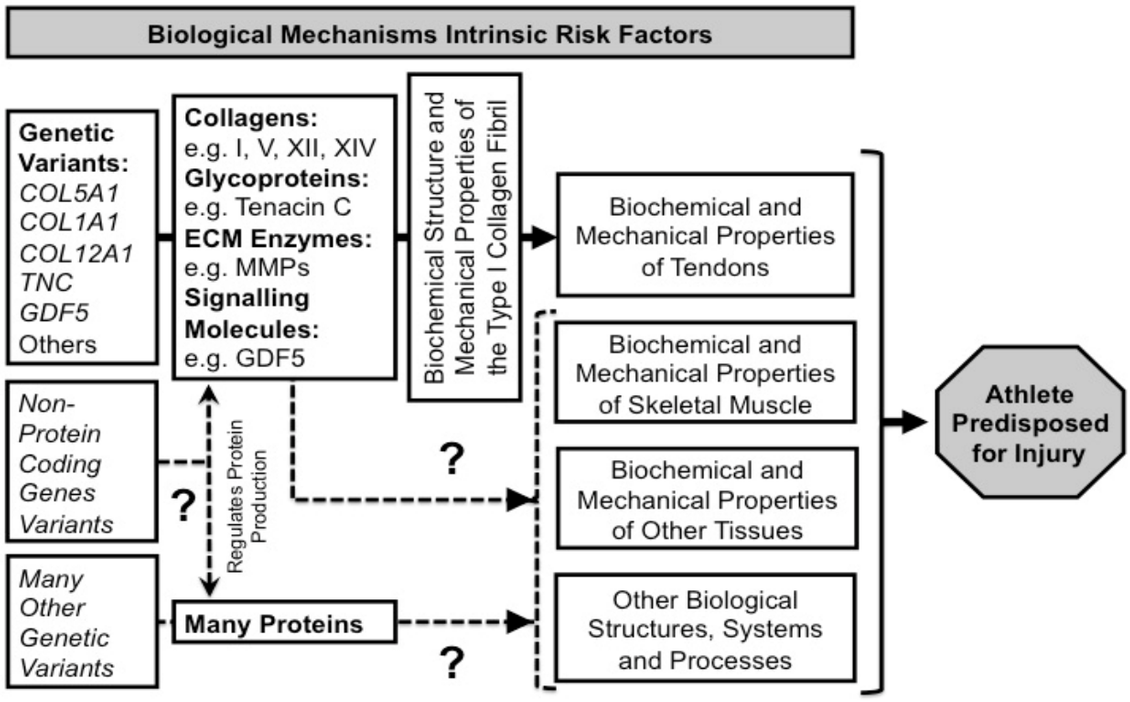

Fig. 2. A schematic illustrating how proposed molecular and biological mechanisms could more accurately describe intrinsic component for Achilles tendon injury risk. In this hypothetical model, inter-individual variations in the biochemical and mechanical properties of the tendon, skeletal muscle and other tissues, as well as, other biological structures, systems and processes cause susceptibility to Achilles tendon injuries. Structural differences and levels of proteins within the tissue cause the inter-individual variations. These differences are in turn partly determined by functional genetic variations within protein-coding and non-coding genes. The predisposed athletes will become a susceptible athlete if exposed to the appropriate extrinsic factors and only become injured (acute injury) or symptomatic (overuse injury) after a specific, usually identifiable, inciting event as illustrated in figure 1.

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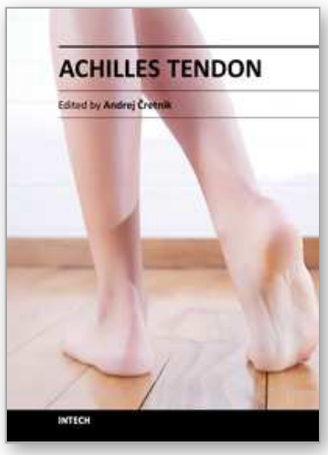

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Achilles tendon has always attracted a great attention. Its disorders include various problems from pain and swelling with bumps to functional impairment or even ruptures. Debates concerning aetiology and optimal treatment are still going on. A lot of efforts and research have already been put on to find the answers to unsolved problems and this book is an attempt to share (some of) these findings to the readers. If only one of the papers helps the therapists or patients in understanding and solving their problems, we will consider that the mission of the book was accomplished.

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