To investigate whether *VEGF* and *KDR* polymorphisms are associated with chronic Achilles tendinopathy and self-reported measurements of tendon pain

by

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DECLARATION

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DEDICATION

I dedicate this thesis to my mother, Mariella Rosa Brazier (06/12/1972 – 22/07/2022) who fought the good fight while battling Multiple Sclerosis. There are no words to describe the impact that you have had on my life. Thank you for inspiring and teaching me to never give up. Riposa in pace.

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LIST OF ABBREVIATIONS

ACL	Anterior Cruciate Ligament
AIDS	Acquired Immune Deficiency Syndrome
Akt	Protein kinase B
ANGPTL4	Angiopoietin like-4
AT	Achilles Tendinopathy
BMI	Body mass index
CGRP	Calcitonin gene-related peptide
Ca^-	Calcium ion
Cl^-	Chlorine ion
cm	Centimetre
Col11a1	Collagen type XI Alpha 1
Col11a2	Collagen type XI alpha 2
Col5a1	Alpha 1 (V) collagen gene
CON	Control group
COX-2	Cyclooxygenase-2
ddNTPs	Dideoxynucleotide triphosphates
DNA	Deoxyribose nucleic acid
ECM	Extra-cellular matrix
EDTA	Ethylenediamine tetra acetic acid
eNOS	Exogenous Nitric Oxide Synthase
EUR	European population
FACITs	Fibril-associated collagens with interrupted triple-helices
FGF-2	Fibroblast growth factor-2
Gln	Glutamine
GCP	Good clinical practice
HIF	Hypoxia inducible factor
Hifa	Hypoxia inducible factor-alpha
His	Histamine
HIV	Human Immunodeficiency Virus
HPALS	Health through Physical Activity, Lifestyle and Sport Research Centre

HWE	Hardy Weinberg Equilibrium
IASP	International Association for pain
IBM SPSS	Statistical Package for the Social Sciences
ICH	International conference of Harmonisation
IL	Interleukin
iNOS	Endogenous Nitric Oxide Synthase
K^+	Potassium ion
KDR	Kinase Domain Receptor
kg	Kilogram
µl	Microlitre
MID	Middle-third of the tendon
m	Metre
mm	Millimetre
MMMP3	Matrix metalloproteinase 3
MMPS	Matrix metalloproteinases
mRNA	Mitochondrial ribose nucleic acid
MTJ	Musculotendinous junction
Na^+	Sodium ion
NK-1 R	Neurokinin-1 receptor
NMDAR1	N-methyl-D-aspartate receptor
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
NSAIDS	Non-steroidal anti-inflammatory drugs
OTJ	Osteotendinous junction
PCR	Polymerase chain reaction
PGE1	Prostaglandin E1
PGE2	Prostaglandin E2
PPI	Present pain index
RA	Rheumatoid arthritis
RPM	Revolutions per minute
RT-PCR	Real-time polymerase chain reaction
SDS	Sodium dodecyl sulphate in distilled, deionized water

SEV	Grades III and IV injuries
sf-BPI	Short-form brief pain inventory
sf-MPQ	Short-form McGill Questionnaire
SNP	Single Nucleotide Polymorphisms
SP	Substance P
SPHK1	Sphingosine kinase 1
SPK1	Serine-arginine rich protein
SRSF1	Phosphorylating seine-arginine rich splice factor
SSISA	Sports Science Institute of South Africa
STREGA	Strengthening the Reporting of Genetic Association studies
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
TEN	Chronic Achilles Tendinopathy/Cases for the affected group
TGF-a	Transforming growth factor TGF-a
TH	Tyrosine hydroxylase
TIPS	Tissue inhibitors of metalloproteinases
TNF-a	Tumour necrosis factor-alpha
TRP	Transient receptor potential
VAS	Visual analogue scale
UCT	University of Cape Town
VEGF	Vascular Endothelial Growth Factor
VEGFA	Vascular Endothelial Growth Factor Alpha
VEGF-1	Vascular endothelial growth factor 1
VEGF-2	Vascular endothelial growth factor 2
VEGFR-1	Vascular endothelial growth factor receptor 1
VEGFR-2	Vascular endothelial growth factor receptor 2
VISA-A	Victorian Institute Sports Assessment Questionnaire

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ABSTRACT

Background: Chronic Achilles tendinopathy (TEN) is prevalent in the sporting population, specifically in sporting codes with a large running component, and presents as swelling, impaired lower limb function, and pain of an insidious onset. Although the mechanisms are unclear, current theories implicate structural changes and neovascularisation in tendinopathy. Vascular endothelial growth factor alpha (*VEGFA)* and its receptor referred to as kinase domain receptor (*KDR*) are key regulators of neovascularisation and can be associated with pain. Common DNA sequence variants within the *VEGFA* and *KDR* genes have previously been associated with musculoskeletal soft tissue injuries, including TEN. The primary aim of this dissertation was to identify whether *VEGFA* and *KDR* variants were associated with (i) the severity of TEN, (ii) TEN ultrasound findings and (iii) self-reported measurements of Achilles tendon pain using multidimensional pain scales.

Methods: One hundred and eighty-five recreational athletes with clinically confirmed Achilles tendinopathy for at least 3 months were recruited from Cape Town, South Africa. The injured and uninjured Achilles tendons were examined using the conventional grayscale ultrasound. Tendinopathy pain was rated by completing the Victorian Institute Sports Assessment -Achilles (VISA-A), short-form McGill Pain Questionnaire (sf- MPQ), and short-form Brief Pain Inventory (sf-BPI) questionnaires. One hundred and ninety-four asymptomatic healthy appropriately matched individuals with no history of tendon injuries were also recruited for this study. Participants were genotyped for the *VEGFA* rs699947 (C/A), *VEGFA* rs2010963 (G/A), *KDR* rs2071559 (G/C) and *KDR* rs1870377 (T/A) polymorphisms.

Results: Although the *VEGFA* and *KDR* variants were not associated with TEN, either independently, or as inferred haplotypes or via allele interactions, (i) the *VEGFA* rs699947 CC genotype ($p = 0.019$, OR: 0.29, 95% CI: 0.12, 0.72) was significantly associated with decreased risk of bilateral TEN, (ii) the A-G *VEGFA* inferred haplotype ($p = 0.005$) constructed from rs699947 and rs2010963 was associated with increased risk of bilateral TEN, (iii) the *KDR* rs2071559 AA genotype ($p = 0.016$) was significantly associated with increased risk of a history of multiple (two ormore) TEN, (iv) the G-T ($p = 0.028$, OR: 0.51; 95% CI: 0.31, 0.85) and A-A ($p = 0.012$) *KDR* inferred haplotypes constructed from rs2071559 and rs1870377 were associated with decreased risk and increased risk of multiple and/or bilateral TEN,

respectively, and (v) the C-G (p = 0.008) and A-A (p = 0.014, OR: 0.36, 95% CI: 0.16, 0.83) *VEGFA* rs699947 and *KDR* rs2071559 allele-allele interactions were significantlyassociated with decreased and increased risk of bilateral or multiple injuries respectively. There were no significant differences in the diameters or the relative number of abnormal ultrasound findings of the injured and uninjured Achilles tendons between the *VEGFA* and *KDR* genotype groups. Finally, there were no significant differences in the VISA-A, sf-MPQ and sf-BPI scores, as well as the subscale scores between the *VEGFA* and *KDR* genotype groups.

Conclusion: The novel findings of this dissertation implicate the *VEGFA* and *KDR* genes, and by implication the potential biological role of the angiogenesis signalling pathway, with bilateral and/or multiple Achilles tendinopathy risk. The investigated variants within these genes however were not associated with tendon diameters, the relative number of abnormal ultrasound findings or self-reported Achilles tendon pain measured using multidimensional pain scales.

PREFACE

…According to Greek mythology, Ἀχιλλεύς or Achilles was the most handsome and mightiest Greek warrior of the civilized world. Achilles' body was mystically protected from harm – a boon of invulnerability was bestowed on him as a child when his mother submerged him into the River Styx. Everything that the magical waters touched became invulnerable, but the water did not wet his heel, leaving it unprotected. During the Trojan War, at the height of his power and martial prowess, Achilles was struck by a poisoned arrow on his vulnerable heel and died. It was this legend that inspired Philip Verheyen in 1693 to describe the tendon located at the heel as the "cord of Achilles" or alternatively in his textbook *Corporis Humani Anatomia*. This led to this tendon being commonly and medically referred to as the Achilles tendon.

Chapter 1

Literature review

1.1 Scope of the Dissertation

The Achilles tendon is situated in the posterior superficial compartment of the lower leg, where it attaches the gastrocnemius and soleus (calf) muscles to the calcaneus (heel bone) allowing for the force generated in the calf muscles to be transmitted to the heal joint (1–3). It is resilient and has a high tensile strength, being able to withstand forces of up to ten times the body's weight and is therefore considered to be the strongest tendon in the body. The Achilles tendon is able to absorb the forces applied to it in order to minimize muscle damage and therefore possess flexibility and elasticity (1,4).

The Achilles tendon is innervated by the sural nerve, with minor contributions from the tibial and peroneal nerves, which govern the pain thresholds experienced by individuals upon injury (4,5). The blood supply to the tendon originates from three sources, namely that of the musculotendinous junction (MTJ), osteotendinous junction (OTJ) and surrounding connective tissues. Unlike the MTJ and OTJ, which are well-perfused, the tendon's mid-portion is not, which could predispose it to injury (1,6,7).

Although there are different types of injuries which can affect the Achilles tendon and surrounding tissues, (4,8) this dissertation will focus on chronic Achilles tendinopathy (TEN), which is common among individuals who participate in physical activities with a significant running component (9–11). Chronic Achilles tendinopathy is a heterogenous condition with pain of an insidious onset being the main presenting complaint and is often unresponsive to treatment (5,12–14). Individuals experience varying degrees of pain which interferes with day-to-day activities (3). The International Association for the Study of Pain (IASP) has defined pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in such damage" (15). The pain experienced by individuals with Achilles tendinopathy, which is currently explained by the central pain modulation (16,17) and several peripheral mechanisms (18–21), is the body's response to a stimulus in order to prevent further damage.

The Victorian Institute Sports Assessment - Achilles (VISA-A) is a well-established validated questionnaire used by clinicians to measure pain experienced in Achilles tendinopathy (22). However, specifically due to its inability to measure pain beyond its effect on physical function, Mkumbuzi (23,24) recently proposed the use of the short-form McGill Pain Questionnaire (sf-MPQ) and the short-form Brief Pain Inventory (sf-BPI) as additional tools to measure pain experienced in Achilles tendinopathy (25,26). The sf-MPQ questionnaire uses an eleven-item sensory (continuous, intermittent and neuropathic) and a four-item affective subscale to measure subjective pain (26). The sf-BPI scale measures pain on how it affects the functioning of individuals during daily activities, which includes general activity, mood, walking ability, work, relationships with others, sleep and enjoyment of life (27). Using these questionnaires, tendon pain was predominantly described as a continuous sensory pain and less frequently as an intermittent or a neuropathic type of sensory pain. Additionally, the pain experienced by these individuals interfered with walking ability, mood, sleep, enjoyment of life (23,24,28) and furthermore, unlike other types of chronic pain, chronic Achilles tendon pain was largely local or peripheral. The peripheral mechanisms of pain includes biochemical and/or structural changes in the tendon, (18–21) such as neovascularisation (29), inflammation (5,30,31) and ion channel abnormalities (32).

Neovascularisation, which is regulated by an anomaly of factors including mechanical loading, is a multiplex process whereby pro-angiogenic factors are produced resulting in the formation and proliferation of blood vessels within tissues (33). Increased neovascularisation has been reported in degenerative and ruptured Achilles tendons (34,35), rotator cuff tendinopathy (36) and ligament injuries (37). The presence of neovascularisation within the Achilles tendon can indicate hypoxic conditions and compromised tissue repair, which is further associated with pain (38–43).

Vascular endothelial growth factor alpha (VEGFA) and its receptor referred to as kinase domain receptor (KDR) or vascular endothelial growth factor receptor-2 (VEGFR-2) are key regulators of neovascularisation. There are five isoforms of the *VEGF* gene, with Vascular endothelial growth factor-alpha (*VEGFA)* displaying the highest angiogenic ability (44,45). An increase in *VEGF*, specifically *VEGFA* expression has been reportedly upregulated amonginjured tendons (46,47) among individuals undergoing recovery in the mid-portion of the Achilles tendon, as well as ruptured or degenerative tendons (11,48). KDR receptors permit the entry of cations through a non-selective membrane known as the transient receptor

channel which regulates the pain. The involvement of the *VEGF-KDR* pathway in the aetiology of the pain associated with chronic Achilles tendinopathy therefore warrants further research (49).

The association of three functional *VEGFA* rs699947 (-2578 C>A), rs1570360 (-1154 G>A) and rs2010963 (-634 G>C), as well as the functional *KDR* rs2071559 (-604 G>A) and rs1870377 (1719 T>A) polymorphisms have previously been investigated with chronic Achilles tendinopathy (50), tendinopathy in volleyball players (10) and ACL ruptures (50– 57). Although the *VEGFA* A-G-G inferred haplotype constructed from rs699947, rs1570360 and rs2010963 was associated with an increased risk of Achilles tendinopathy in a combined South African and British cohort as well as in a South African cohort, however, it was not associated with a British cohort when analysed separately (50).

It is not unusual for initial genetic associations with multifactorial conditions, including musculoskeletal soft tissue injuries, not to be repeated in follow-up studies (58). Although there are several reasons for this, heterogeneity of the clinical phenotype is one possibility. This and other published tendinopathy cohorts consisted of unilateral, bilateral, single and multiple injuries, grades I to IV injuries and different injured regions within the tendon. The association of the functional *VEGFA* and *KDR* polymorphisms with regards to severity, region and type of Achilles tendon pain has not previously been investigated. Therefore, a candidate gene case-control genetic association study approach was used in Chapter 3 of this dissertation to further investigate the association of *VEGFA* and *KDR* polymorphisms with chronic Achilles tendinopathy in a combined larger cohort consisting of two previously described South African physically active groups (24,50). Initially the association of these polymorphisms with respect to the larger cohort was investigated irrespective of severity, number and region of injury. This was followed by investigating the association with respect to severity, number and region of injury. In addition, a cross-sectional genetic association study approach was used in Chapter 4 to investigate the association of these polymorphisms with ultrasound and multidimensional pain measurements in participants with chronic Achilles tendinopathy. The two results chapters (Chapters 3 and 4), will be preceded by a literature review (Chapter 1) and methodology (Chapter 2), followed by a summary and discussion of the findings of this dissertation in Chapter 5. The following sections of this chapter will review the anatomy and molecular structure of the Achilles tendon (Sections 1.2 and 1.3), chronic Achilles tendinopathy (Section 1.4), measurement and mechanisms of tendon pain (Section 1.5), regulatory pathways and

Achilles tendinopathy (Section 1.6), and finally, the association of *VEGFA* and *KDR* with respect to musculoskeletal soft tissue injuries (Section 1.7).

1.2 Anatomy of the Achilles tendon and Surrounding Tissues

The Achilles tendon is regarded as the strongest and thickest tendon within the human body as it is able to withstand forces of up to ten times the body's weight (59). The tendon ranges approximately between 11 and 26 centimetres in length and has an average diameter of between 4.5 and 9 centimetres, has a rounded upper appearance, is relatively flat (60), thinner at its midsection and rounded above the calcaneus. It is located in the posterior superficial compartment of the lower leg, whereby it attaches to the calf muscles, namely that of the gastrocnemius and soleus with minor contributions to the plantaris, via the musculotendinous junction (1,59) (Figure 1.1).

The gastrocnemius and the soleus muscles form a triceps surae which assists with plantarflexion of the ankle joint via the Achilles tendon (1) The plantaris is a tiny and slender vestigial muscle originating from the popliteal surface of the femur and is placed distally across the calf muscles. It can be found in the medial aspect of the Achilles tendon; however, it can be absent in up to 8% of individuals (4,46) (Figure 1.1).

The calcaneal insertion is formed from the osteotendinous junction (OTJ), sesamoid fibrocartilage near the dorsal deep surface of the tendon, a fibrocartilage layer which covers the periosteum and Kager's fat pad. Kager's fat pad is made of a mass of adipose tissue forminga triangular region. Imaging has associated the fat pad with the Achilles tendon, flexor hallicus longus tendons and the fat surrounding the calcaneus (2). During dorsiflexion, the bursal wedge relaxes and permits movement by altering its shape and synovial folds. Fibrous connections which bind the fat to the Achilles tendon, stabilize the region and protects the blood vessels entering the tendon, while decreasing the pressure changes which cause injury (61). The fibrous connections which attach the fat pad to the tendon enhance its proximal stability, while bursae are arranged distally and superficially deep along the tendon. The fat pad is moveable and protrudes into the retro-calcaneal bursa during contraction (plantarflexion) and retraction (dorsiflexion) of the tendon, promoting free movement. Adding to this, the flexor hallicus longus is situated deep to the fascia on the tendon's anterior surface (2) and is prone to

increased stress which is often subject to overuse injuries referred to as enthesopathies (62,63). The site where the Achilles tendon attaches to the calcaneus with the retrocalcaneal bursa intervening proximal to the attachment aids in injury prevention (2).

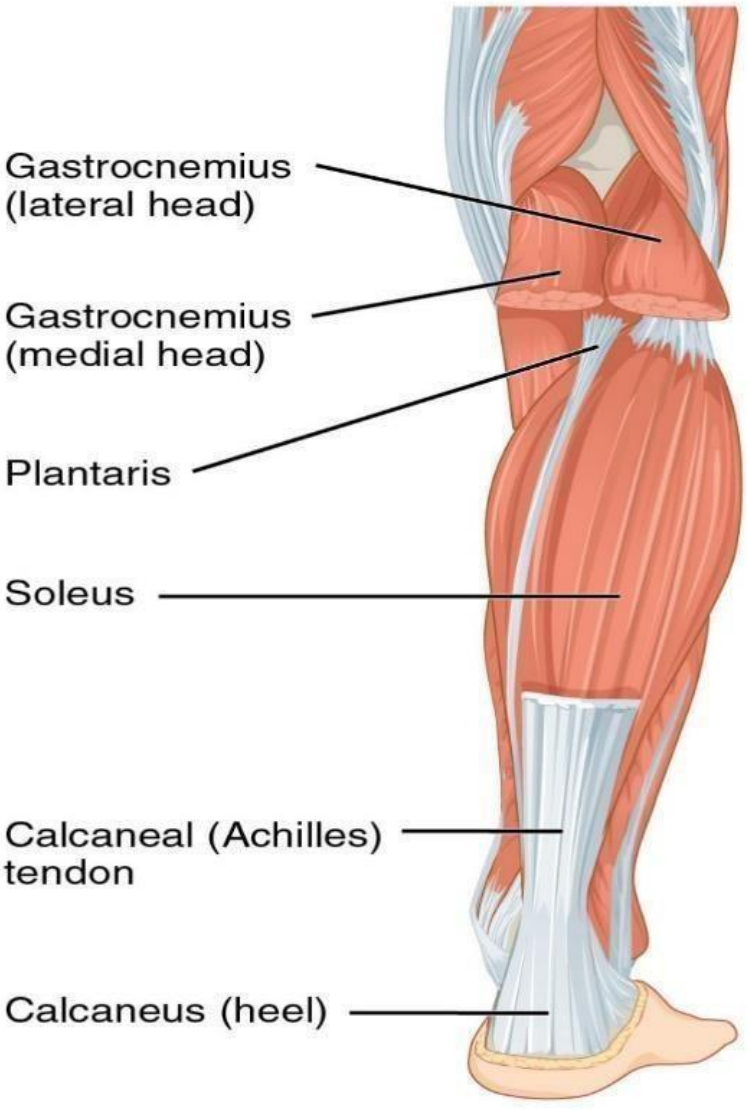

Figure 1.1: Illustration of the soleus, gastrocnemius, Achilles tendon and its insertion onto the calcaneus. Contributed by OpenStax College, 2017. (Creative Commons Attribution 3.0 license) https://www.ncbi.nlm.nih.gov/books/NBK499917/. Retrieved on 5 October 2023.

The Achilles tendon has a rotation capacity of up to 90 degrees as it moves away from its proximal to distal attachment in order to produce an area of concentrated stress, while its medial attachment is superficial and lateral side is largely deep. The arrangement of the tendon aids in its elongation and release during locomotion (60). The fibres of the tendon are occasionally vertically aligned as they have a noticeable spiral appearance upon fusing with the calf muscles. The degree of rotation in the leg is determined by the fusion of the muscles and therefore, there will be better rotation when the fibres are more distally located (64).

Tendons receive their sensory innervation from superficial and deep nerves. The Achilles tendon is innervated by nerves from surrounding muscle tissues and cutaneous nerves. The fibres from these nerve-endings form a longitudinal plexus which innervates the afferent fibres close to the OTJ with receptors namely that of Type I Ruffini corpuscle pressure receptors, Type II Vater-Pacinian corpuscles sensitive to movement, Type III Golgi tendon mechanoreceptors and Type IV free nerve endings which assist with pain sensitization (4,65).

The Achilles tendon receives its blood supply from namely the MTJ, OTJ and surrounding connective tissues (1,65). The blood supply to the tendon is dependent on age and is likely to decline with age (9). The posterior tibial artery aids in perfusing peritendinous tissues and is considered to be the primary artery of perfusion (4). The blood supply to the tendon's mid-portion originates from the paratenon running transversely towards the tendon, however, these vessels do not enter the surrounding collagen (1). Therefore, the vascularity of its mid-portion is relatively poor as blood enters primarily into the anterior space and at sites of friction, torsion or compression whereby blood supply is compromised (66). The paratenon within the Achilles tendon is highly vascularised and receives minor contributions from the fibular and posterior tibial arteries (6,65). Due to hypo-vascularisation hypothesized at the tendon's mid-portion, more injuries have been reported in this area (4), however factors such as the forces applied, and tendon biomechanics could add to its predisposition to injury at its mid-portion (66).

1.3 Molecular Structure of the Achilles tendon

Healthy Achilles tendons are white in appearance and have a hierarchical structure consisting predominantly of type I collagen fibrils (66) (Figure 1.2). Collagen fibrils, which are the smallest structural units of tendons, aggregate into larger structural units termed collagen fibres. These fibres are enclosed by areolar connective tissue sheaths also known as the

endotenon and aggregate into larger structures termed fascicles, which in time collectively form the tendon. Each fascicle is surrounded by an endotenon or inter-fascicular matrix which permits blood vessels, lymphatic vessels and nerve fibres to enter the tendon. The entire tendon is surrounded by an inner-connective tissue sheath termed the epitenon and an outer-layer of loose connective tissue termed the paratenon. Fluid is situated in-between the epitenon and paratenon to reduce the effects of friction (46,66,67). The collagen fibres are densely arranged as parallel bundles within the structural layers of the tendon and arranged in a manner ensuring limited macroscopic failure (4,46,67,68).

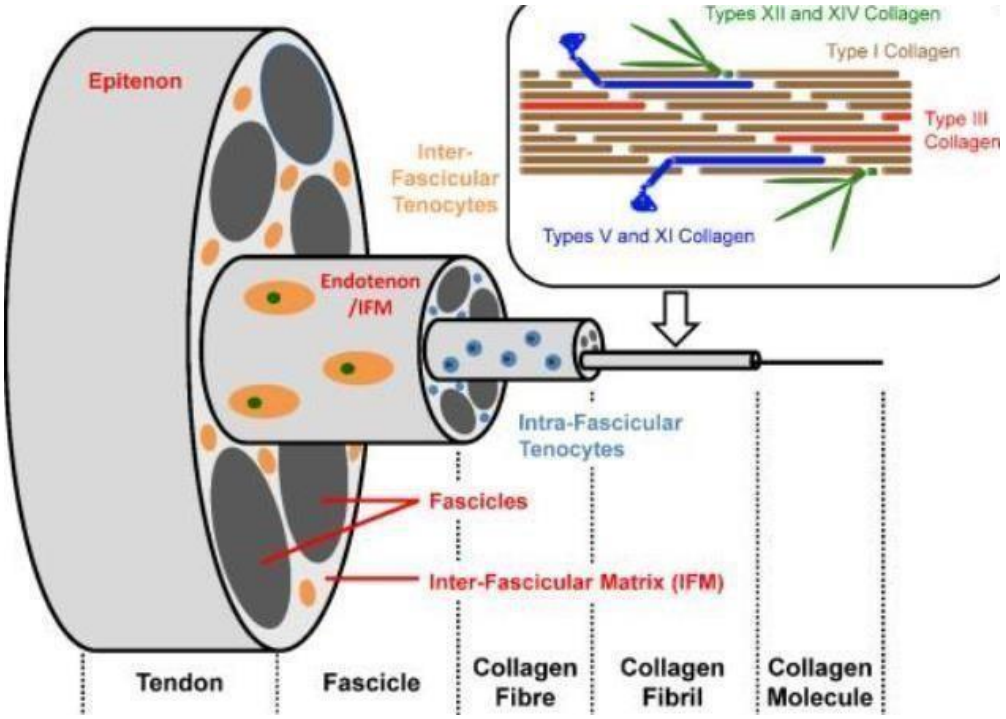

Figure 1.2: Schematic diagram of a tendon in cross-section. Collagen fibrils are bundled into fascicles containing vessels, lymphatics and nerves. The fascicles are grouped together, surrounded by epitenon and from the gross structure of the tendon, which is further enclosed by paratenon (58).

The collagen fibril consists predominately of type I collagen, which has a triple helical structure consisting of two a1(I) and one a2(I) polypeptide chains (66) (Figure 1.2). The fibril contains small amounts of other functionally important fibrillar collagens such as types III and V collagen (69). Type III collagen is a homotrimer consisting of three a1(III) chains, while the major isoform of type V collagen consists of two a1(V) and one a2(V) chains. Different collagen types, known as fibril-associated collagens with interrupted triple-helices (FACITs) are associated with the fibril. Important FACITs include types XII and XIV collagen (69–72).

The collagen and elastin fibres, which make up approximately 2% of the tendon's dry weight, and several other non-fibrous forming proteins within tendons are embedded in the ground substance consisting of proteoglycans, glycosaminoglycans and water (72). Collagen, elastin, non-fibrous forming proteins, glycoproteins and proteoglycans within tendons are produced as tenocytes and tenoblasts and constitute an estimated 95% of the tendon's cellular content (72,73). The spindle shaped tenocytes are predominately found within the fascicles between the collagen fibres, while metabolically active tenoblasts are found within the inter-fascicular matrix (3,67,70).

In addition to the tensile forces, the OTJ of the Achilles must resist compressive forces and therefore has more of a fibrocartilage structure (4,70). Both type I and cartilage-specific type II collagen fibrils are synthesised together in fibrocartilaginous tissues (59,74). The major collagen type in the type II collagen fibril is type II collagen with trace amounts of type XI collagen. Different proteoglycans, such as aggrecan, which bind more water to resist compressive forces are also produced in the fibrocartilage (75). Tendons are metabolically active and adapt to external stimuli, however they have a low basal metabolic rate (66) that causes postural tension without ischemia. This decreases its rate of healing, thereby making recovery a complex and slow process often associated with pain (59).

1.4 Chronic Achilles Tendinopathy

Achilles tendinopathy is a common ankle and foot overuse injury (76) with presenting symptoms such as pain (5), swelling and impaired performance. The pain associated with tendinopathy is the main presenting complaint, has an insidious onset, is often unresponsive to treatment and therefore requires prolonged rehabilitation as well as an acumination of treatment strategies (5,12,14,34). Individuals with Achilles tendinopathy experience varying degrees of

pain which interferes with day-to-day activities (5). Individuals who participate in sports with a running and jumping component are at an increased risk of Achilles tendinopathy as it has been reported that up to 9% of recreational runners and up to 5% of athletes who run are affected by Achilles tendinopathy (77). It is also common in the sedentary population with an estimated 6% of the population being affected (78).

The pain experienced in Achilles tendinopathy is rarely associated with rest periods, it is more prevalent when there is excessive use of stored energy, especially during running or when rising in the morning (40). Excessive loading of the tendon during high intensity interval training activities can result in intensified pain (79). The pain experienced in this condition is the most unpleasant during warm-up periods and less apparent during exercise, however it tends to reappear during exercise (80,81). This condition does not have a definitive triggering event, however it can be increased when the force and load applied to the tendon increases during training, depending on the intensity and type of training programme (18,32,81,82). The pain can also be associated with tenderness to palpation (9,50,53,54) and radiates outwards to other neighbouring structures, resulting in lower limb pain and reduced functioning of the calf muscles (18,80,81).

1.5 Measurement and Mechanisms of Tendon Pain

Pain has been defined by the International Association for the Study of Pain (IASP) as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in such damage" (15). The pain experienced by individuals with chronic Achilles tendinopathy is the body's response to a stimulus in order to prevent further damage, which is currently explained by the central pain modulation (16,17) and several peripheral mechanisms (19–21,83).

The Victorian Institute Sports Assessment-Achilles (VISA-A) is a well-established and validated questionnaire used by clinicians to measure pain in Achilles tendinopathy (22). Due to its inability to measure pain beyond its effect on physical function, Mkumbuzi (24) proposed the use of the short-form McGill Pain Questionnaire (sf-MPQ) and the short-form Brief Pain Inventory (sf-BPI) as additional tools to measure pain experienced in tendinopathy.

The validated sf-BPI scale, which has been used in various conditions, including cancer, osteoarthritis, Achilles tendinopathy, spinal cord injuries, HIV, AIDS and many musculoskeletal injury related conditions (15,84–86), measures the effect of pain on various daily activities and enjoyment of life. These include general activity, walking ability, work, sleep, relationships with others, mood and enjoyment of life (27). Using this questionnaire, Achilles tendon pain was reported to have interfered with walking ability, sleep, mood and enjoyment of life (24).

The sf-MPQ questionnaire uses: (i) an eleven-item sensory subscale and (ii) a four-item affective subscale consisting of adjectives commonly used to describe subjective pain. Of the eleven-item sensory subscale, words such as 'aching', 'tender', 'throbbing', 'cramping', 'gnawing' and 'heavy' describe continuous pain, while 'sharp', 'shooting', 'stabbing' and 'splitting' describe intermittent pain and 'hot-burning' describes a neuropathic type of pain (26). Tendon pain was predominantly described as a continuous sensory pain (aching, tender, throbbing) and less frequently as an intermittent (sharp) or a neuropathic type of sensory pain. The four-item affective subscale includes adjectives such as 'sickening', 'punishing-cruel', 'fearful' and 'tiring-exhausting'(23,24).

Unlike other types of chronic pain, these questionnaires described chronic Achilles tendon pain as predominately local or peripheral. The peripheral mechanism of pain includes biochemical and/or structural changes in the tendon (18,19,21) such as (i) inflammation (5,30,31,87), (ii) ion channel abnormalities (32) and (iii) neovascularisation (29). The following section will review the current literature regarding these structural and biochemical changes associated with tendon pain.

1.5.1 Inflammation

Tendinopathy was previously classified as tendinitis since it was believed that it was primarily an inflammatory condition (5,78,82,88). Earlier histological studies of tendinopathic tissues showed regions of increased concentrations of glycosaminoglycans within the ground substance, disorganization of collagen and areas of hyper or hypo-cellularity and neovascularisation with a noted absence of inflammatory cells or markers (89). Although the term tendinopathy was adopted, the role of inflammation associated with tendon pain has not been ignored as subsequent immunohistochemistry and gene expression studies have correlated

inflammation in the healing process (11,90). Studies have identified the presence of immune cells such as macrophages, T-cells, natural killer cells and mast cells in non-ruptured TEN and the significant role of apoptosis in tendon injury. Therefore, it is plausible that inflammation is common in TEN and should be further investigated (87).

In recent years, inflammation has become associated with a broad range of diseases as it aids tissue repair and follows a cascade of cellular and microvascular pathways (90). Inflammation is an important feature of tendon disease progression and development. Individuals with TEN present with elevated levels of expressed inflammatory cells (32,91), including CD14, CD68 as well as the activation of pathways involving NF-kb, prostaglandins, neuropeptides, growth factors, interferons and STAT-6 (87). The Achilles tendon presents with elevated levels expressing pro-inflammatory and stromal fibroblast markers following cytokine stimulation. These molecular and inflammatory mechanisms can be due to short-term inflammation and neovascularisation (5).

There are several types of cells which respond as inflammatory mediators such as that of interleukins, prostaglandins (PGE1 and PGE2) and nitric oxide (NOS). PGE2 has been reportedly increased among 50% of individuals with tendinopathies. When the Achilles tendon is subjected to cyclic stress, IL-1, IL-6, IL-1b, TNF and PGE2 cause an increase of COX-2, MMP-1, MMP-3 and MMP-19 which in turn degrades the matrix, *VEGF*, cytosolic phospholipase-A2 and stress-activated protein kinase (87). COX-2 expression is increased by the presence of neurotransmitters, pro-inflammatory cytokines, growth factors as well as other hormones. When there is inflammation and prostanoids, tissue injury is evident as prostanoids induce inflammation and mediate the body's sensitivity to pain. Substance P and CGRP play a vital role in pain modulation and inflammation, while neuropeptides exert trophic effects and cause inflammation. Genes associated with angiogenesis, such as *VEGF* are upregulated in injured tendons, whereby inflammation occurs due to the absence of clearing the apoptotic cells and inflammatory molecules (87).

1.5.2 Ion channel abnormalities

Channel proteins within the plasma membrane extend to the cytosol of the cell and selectively permit ions to pass through their pores. Proteins which transport inorganic ions are referred to as ion cannels(92). These channels permit the entry of ions at a rate 10^5 times faster than other

carrier proteins, however, this type of transport is passive. Ions such as Na^+, K^+, Ca^{2+} and Cl^- diffuse down electrochemical gradients via a lipid layer. The modulation of these channels is important for many cell functions such as receiving, conducing and transmitting information within neurons (92).

There are no clear mechanisms which explain the pain experienced among individuals with chronic tendinopathies (5), thereby impacting the prescription of the most appropriate treatments as an estimated 10% of individuals with TEN will have a lifetime prevalence of the tendinopathy (38). Microscopic examination from tissue biopsies have shown collagen disorientation, disorganisation and fibre separation in those with TEN (93), however, not all individuals with these symptoms will report pain or be clinically diagnosed. Pain experienced in tendinopathies can be physiological or pathophysiological. Physiological pain reflects the activation of primary nociceptors, whereas the latter is associated with functional changes within the nervous system (32,73).

Allodynia and primary hyperalgesia affect the sensation of primary nociception related to pain. Tenocytes are attached end-to-end in ion channels, permit communication and perform functions such as cell volume control, osmoregulation and calcium signalling (94) as they join to the cytoskeleton and extracellular structures. These channels are able to sense an incoming stimulus and communicate with neurons, however when there is abnormal functioning, ion channels will be adversely affected. This is due to the decrease in pH and increase in acidity as a result of excess lactate in tendinopathic conditions (95–97).

Ion channels are required in order to activate neuronal pathways as their expression in tenocytes and afferent nerves can be altered due to repeated activation (98). These channels transduce noxious stimuli and permit action potentials to the central nervous system from the peripheral site (99). The ion channels present in tenocytes could explain the pain experienced as they would remain closed when there is no stimulus, however, when there is prolonged stimulation many of these channels close (32,100). Therefore, when ion channel abnormalities arise, communication between cells, such as tenocytes for the purpose of this dissertation, is not effective and can compromise several signalling pathways including blood vessel formation leading to neovascularisation (11,99) thereby collectively impacting pain (32).

1.5.3 Neovascularisation

As reviewed in section 1.3, healthy tendons consist of a hierarchical structure packed with parallel bundles of type I collagen fibrils surrounded by ground substance. Structural changes in TEN includes tendon thickening as a result of an increased cross-sectional area, loss of parallel fibrillar arrangement, water retention and neovascularisation (66,101).

Neovascularisation, which is regulated by an anomaly of factors including mechanical loading (102,103), is a multiplex process whereby pro-angiogenic factors are produced resulting in the formation and proliferation of blood vessels within tissues (33). Increased neovascularisation has been reported in degenerative tendons, ruptured Achilles tendons (34,35), rotator cuff tendinopathy (36,92) and ligament injuries (37). The presence of neovascularisation in the Achilles tendon can indicate hypoxic conditions and compromised tissue repair, which is further associated with pain (38–41,43).

VEGF and its receptor referred to as KDR or vascular endothelial growth factor receptor-2 *(VEGFR-2)* are key regulators of neovascularisation. An increase in *VEGF*, specifically *VEGFA* expression has been reported in tendon cells (44), among individuals undergoing recovery in the mid-portion of the Achilles tendon (11), as well as ruptured or degenerative tendons (11,48). The KDR receptor permits the entry of cations through a non-selective membrane known as the transient receptor channel, which regulates the pain experienced among individuals and will be further reviewed. There are many feedback systems within the ECM of tendons and therefore it is not surprising that the genes functioning in the ECM can affect pain (49,104).

1.6 Regulatory Pathways and Achilles Tendinopathy

The investigation of molecular mechanisms which are regulated during tendon injuries, specifically for the purpose of this dissertation are vital in understanding the pathogenesis of Chronic Achilles tendinopathy. The metabolically active characteristics of tendons permit them to respond to environmental stimuli, and with mechanical loading an increase in the expression of several signalling molecules can be activated, such as several cytokines, *VEGF* and transforming growth factor (35,105,106). Studies have demonstrated that cyclic strain in *in-vitro* tendons upregulated the production of angiogenic molecules such as angiopoietin like-4

(*ANGPTL4*), fibroblast growth factor-2 (*FGF-2*), cyclooxygenase-2 (*COX-2*), sphingosine kinase-1 (*SPHK1*), transforming growth factor (*TGF-a*) and *VEGFA* (29,106). An increase in vascularity was observed after mechanical loading and in symptomatic Achilles tendons (92,96,106); whereas normal healthy tendons were relatively avascular in structure (35). Although several molecules have been identified this review will focus on *VEGFA*.

Although VEGF is produced during embryogenesis, endogenous VEGF is negligible in healthy adult tendons as the metabolic demand of the tendon is low. *VEGF* expression within the tendon is regulated by hypoxia, inflammatory cytokines, nerves and the mechanical load applied to the tendon. Nerve growth factors upregulate VEGF which is essential for growth. Adding to this, stressors applied to the body increases the production of VEGF, which promotesangiogenesis at earlier stages of tendon healing in order to facilitate the exchange of nutrients and waste (29,35).

VEGFA and its role in the development of trigeminal neuropathic pain has been extensively studied by Lee *et al.,* (2019). *VEGFA* is a key mediator of endothelial cell mitogenesis, angiogenesis and vascular permeability. The gene plays a significant role in neurobiological, neurotrophic and neuroprotective activities within the nervous system (32). Studies conducted on nerve injuries reported an upregulation of *VEGFA* following injury and when inhibited, reduced allodynia in the sciatic nerve (11,104). The importance of *VEGF* in tendon repair has been highlighted, as following injury, alpha granules release numerous growth factors such as VEGF, FGF, IGF, PDGF and TGF-beta (29,35). The role of *VEGF* in pathogenesis and healing as well as its splice variants as possible treatment strategies to enhance angiogenesis stimulation has been reported (42,105,107).

The VEGF family of proteins includes that of VEGFA, VEGFB, VEGFC, VEGFD, Placental growth factor, virus encoded VEGFE, and VEGFF. However, VEGFA is the most potent stimulator of angiogenesis both in its endogenous and exogenous form (49). *VEGFA* is a potent pro-angiogenic agent and a key mediator of neovascularisation, which is associated with pathogenic diseases. Therefore, research into anti-*VEGF*-related drugs are aimed at attenuating neovascularisation in age-related macular degeneration. Despite some studiesopposing *VEGF*, there has been increasing evidence of its role in neuroprotection and nociception. Its role in the pathophysiology of pain has not been fully understood and therefore warrants further research(45,108). VEGFA and VEGFB have been reported to bind

with two different tyrosine kinase receptors, namely that of VEGFR1 and KDR which are expressed in nociceptors. VEGFA exists in alternative splice forms due to the differences on the serine-arginine rich protein (SPK1) which is responsible for phosphorylating the seine-arginine rich splice factor (SRSF1),thereby increasing the amounts of VEGFA (45).

In conditions such as osteoarthritis, VEGF mediates cartilage degeneration and increases the activity of neutrophils and macrophages, which in turn increases the levels of cytokines thereby further evoking pain. When *VEGF* signalling is inhibited, decreased levels of pain are experienced among individuals (11,49,109). VEGF therapies which target ligands or receptors of the gene have shown to reduce pain. *VEGF* was found to be augmented in serum and correlated to TNF-a and other pro-analgesic cytokines, which reduce the expression of *VEGF*. The role of *VEGF* in the relief of pain is most commonly studied among neuropathic pain as opposed to other types of pain. *VEGFA* is neuroprotective and when neutralized, increased pain was also observed (83,110). It is important to consider the roles which vascular and neuronal factors exhibit, such as in angiogenesis, vascular activation and signalling for cell proliferation, collagen organization and pain. Upregulated levels of VEGF can lead to abnormal vasculature, angiogenic pressure and hypoxia, as vessels would not be effective to exchange nutrients (56,111,112). It is therefore not surprising that the role of these key genes have been evaluated in understanding the genetic contribution to injuries.

1.7 The Association of *VEGFA* and *KDR* with Musculoskeletal Soft Tissue Injuries

VEGFA gene consists of nine exons and is located on chromosome 6p21.1 (113,114). It contains proximal and distal splicing sites (114)within the terminal exon, resulting in the production of pro- and anti-angiogenic *VEGFA* isoforms respectively (https://www.ncbi.nlm.nih.gov/gene/7422). The association of three functional polymorphisms at positions -2578 (rs699947 C>A), -1154 (rs1570360 G>A) and/or -634 (rs2010963 G>C) within the *VEGFA* promoter with respect to chronic Achilles tendinopathy and other musculoskeletal soft tissue injuries have previously been investigated (10,51,53,56). The -2578 C, -1154 G and -634 C *VEGFA* alleles have all been linked with an increased *VEGFA* expression (115,116).

Moreover, the *VEGFA* A-G-G inferred haplotype constructed from rs699947, rs1570360 and rs2010963, was significantly associated with an increased risk of Achilles tendinopathy in a

South Africa cohort with participants of self-reported European ancestry as well as a combined South African and British cohort (50). The inferred haplotype was not however associated with Achilles tendinopathy when the British cohort was analysed separately. In addition, none of the three polymorphisms were independently associated with Achilles tendinopathy in the combined or individual cohorts. For the purposes of this dissertation, it should be noted that the Achilles tendinopathy cases evaluated in these studies consisted of participants with singleand multiple, as well as unilateral and bilateral injuries. The *VEGFA* rs699947 polymorphism and two other *VEGFA* polymorphisms (-460, rs833061 T>C and +936, rs3025039 C>T) were not associated with patellar, shoulder nor Achilles tendinopathy (54,56) in Brazilian volleyball players (10) (Figure 1.3).

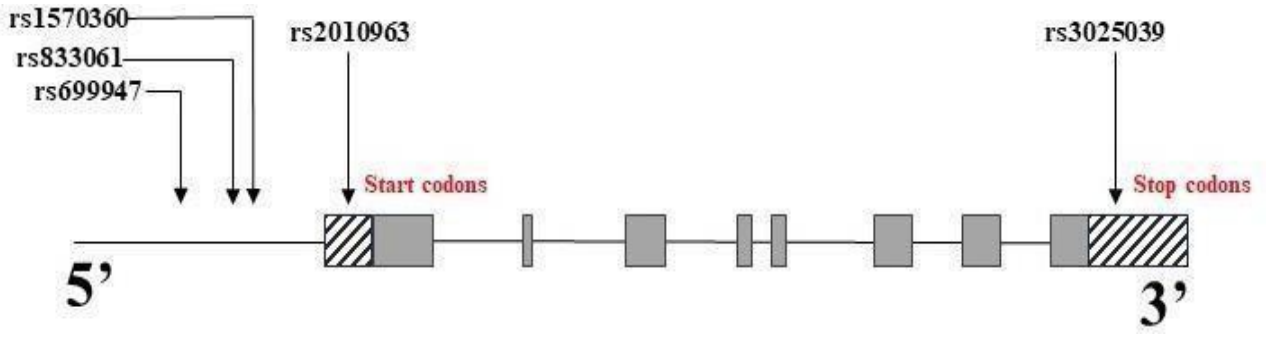

Figure 1.3 Schematic representation (not drawn to scale) of the *VEGFA* gene depicting the rs699947 (-2578 C>A), *VEGFA* rs1570360 (-1154 G>A), rs2010963 (-634 G>C), rs833061 (-460 T>C) and rs3025039 (+936 C>T) polymorphisms. Exons are indicated as grey boxes; introns are indicated as horizontal lines and untranslated regions are indicated as hatched boxes. All the information used to construct the figure above was obtained from databases hosted by the national centre for biotechnology information (NCBI) (https://www.ncbi.nlm.nih.gov/) and Ensemble (https://www.Ensemble.org/index.html).

The *VEGFA* locus was evaluated in a combined analysis consisting of South African, Polish, Australian and Swedish participants, 912 cases and 765 controls self-reported with European ancestry REF. The *VEGFA* A-A-G and A-G-G inferred haplotypes constructed from rs699947

(C/A), rs1570360 (G/A) and rs2010963 (G/C) were significantly associated with a decreased risk of ACL ruptures. In addition, the rs2010963 CC genotype was associated with increased risk of ACL ruptures (51). These *VEGFA* polymorphisms were however not associated with ACL ruptures in a South African population consisting of participants with self-reported Mixed Ancestry (57). Finally, the rs699947 CC and rs35569394 CC genotypes had an eighteen bp ins/del polymorphism at nucleotide -2549 of the promoter. DD genotypes were associated with decreased risk of ACL ruptures in an Indian cohort, while the A-I inferred haplotype constructed from the two polymorphisms was associated with increased risk (56).

The human kinase insert-domain receptor (*KDR*) gene contains thirty exons and is located on chromosome 4q12 (https://www.ncbi.nlm.nih.gov/gene/3791). The association of two functional polymorphisms at positions -604 (rs2071559 G>A) of the *KDR* promoter and at position +1719 (rs1870377 T>A) within exon 11 with respect to chronic Achilles tendinopathy and other musculoskeletal soft tissue injuries have previously been investigated (10,50,51,53,57). The G-allele of rs2071559 within the *KDR* promoter results in decreased gene expression (23,117–120), while the A-allele of *KDR* rs1870377 within exon eleven has aHis to Gln substitution at amino acid position 472. This non-synonymous substitution effects the efficiency of VEGF binding to KDR (10,117,118).

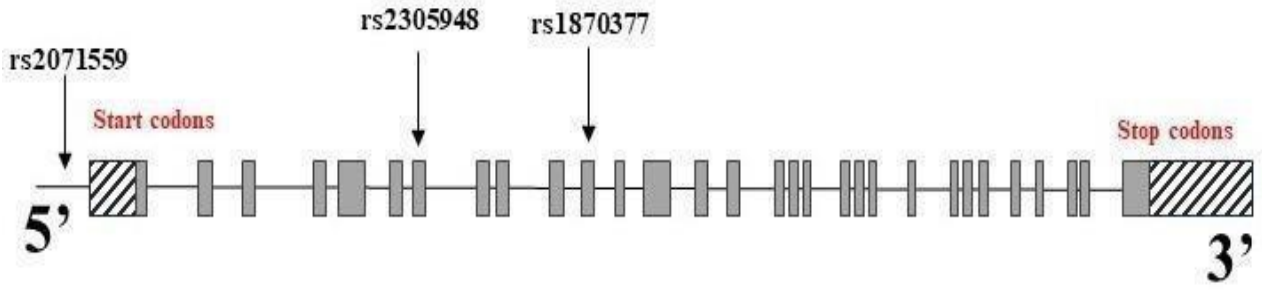

Figure 1.4 Schematic representation (not drawn to scale) of the *KDR* gene depicting the rs2071559 (-604 G>A), rs1870377 (+1719 T>A) and rs2305948 (+1192 G>A) polymorphisms. Exons are indicated as grey boxes; introns are indicated as horizontal lines and untranslated regions are indicatedas hatched boxes. All the information used to construct the figure above was obtained from databases hosted by the national centre for biotechnology information (NCBI) (https://www.ncbi.nlm.nih.gov/) and Ensemble (https://www.Ensemble.org/index.html).

None of these *KDR* polymorphisms were independently or when part of an inferred haplotype associated with Achilles tendinopathy in the combined or individual cohorts (50). The *KDR* rs2071559 (G/A) and rs1870377 (T/A) polymorphisms, together with a third polymorphism (rs2305948, +1192 G>A), was however associated with modulating the risk of patellar,shoulder and Achilles tendinopathies in Brazilian volleyball players. Specially the rs2305948 GG genotype was associated with increased risk of tendinopathy, while the C-G-A and C-A-T inferred haplotypes constructed from rs2071559, rs2305948 and rs1870377 were associated with decreased risk (10).

The GG *KDR* rs2071559 genotype, as well as the G-T inferred haplotype constructed from rs2071559 and rs1870377, was associated with increased risk of ACL ruptures in male participants with self-reported Mixed Ancestry, while the A-A inferred haplotype was associated with reduced risk in males and all participants (53). None of these *KDR* polymorphisms were however independently or when part of an inferred haplotype associated with ACL ruptures in a combined analysis consisting of South African, Polish, Australian and Swedish participants of self-reported European ancestry (51). Although, the *VEGFA* inferred haplotypes constructed from the three functional polymorphisms have been reported to modulate the risk of chronic Achilles tendinopathy (50) the association of the *VEGFA* and *KDR* polymorphisms with ultrasound abnormalities and multidimensional pain measurements in participants with chronic Achilles tendinopathy had no association (24).

1.8 Aims and Objectives

Therefore, the aim of this dissertation was to investigate whether *VEGFA* and/or *KDR* polymorphisms were associated with (i) with number, region and severity of chronic Achilles tendinopathy, as well as (ii) with ultrasound abnormalities and (iii) multidimensional pain measurements in participants with chronic Achilles tendinopathy.

Specifically:-

- a candidate gene case-control genetic association study approach was used to further investigate the association of three functional *VEGFA* rs699947 (-2578 C>A), rs1570360 (-1154 G>A) and rs2010963 (-634 G>C), as well as the functional *KDR* rs2071559 (-604 G>A) and rs1870377 (1719 T>A) polymorphisms with chronic Achilles tendinopathy

irrespective of severity, number and region of injury in a combined larger cohort consisting of two previously described South African physically active groups (Chapter 3).

- investigating the association of the *VEGFA* and *KDR* variants specifically with severity, number and region of injury (Chapter 3).
- a cross-sectional genetic association study approach was used to investigate the association of these polymorphisms with ultrasound abnormalities and multidimensional pain measurements in participants with chronic Achilles tendinopathy (Chapter 4).

Chapter 2

Methodology

2.1 Study design

This case-control (objectives 1 and 2 presented in Chapter 3) and cross-sectional genetic association (objectives 3 and 4 presented in Chapter 4) study was conducted in compliance with the STREGA (Strengthening the Reporting of Genetic Association studies) initiative which is a further extension of the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) statement (121,122). Approval for this study was obtained from the Research Ethics Committee of the Faculty of Health Sciences within the University of Cape Town (UCT) (HREC REF 279/2016; Appendix A).

2.2.1 Participants

A total of 183 cases (TEN) and 194 controls (CON) were previously recruited for this study from sporting clubs within the greater Cape Town area as well as sports and exercise medicine clinics and other clinical practices (Appendix B) (24,50). The data from 81 TEN and 74 CON participants were included from the study conducted by Mkumbuzi (24) and a further 104 TEN and 120 CON participants and their data was included from the study conducted by Rahim (50). All participants were of self-reported European ancestry. A sample size of approximately130 cases and 130 controls was required for this study to have at least 80% power at the 95% confidence level, assuming an odds ratio of at least 2 and allele frequencies greater than 10% (QUANTO v1.2.4 (http://biostats.usc.edu/software)).

Inclusion criteria

In both previously published studies (24,50), the TEN participants reported a history of posterior lower limb pain in the Achilles tendon or the area surrounding the Achilles tendon for a duration of at least 6 months and additionally fulfilled at least one of the following diagnostic criteria: (1) early morning pain over the mid-portion of the Achilles tendon, (2) earlymorning stiffness over the mid-portion of the Achilles tendon, (3) a history of swelling over

the Achilles tendon area, (4) tenderness to palpation of the mid-portion of the Achilles tendon, (5) palpable nodular thickening over the affected Achilles, and/or (6) movement of the painful area in the Achilles tendon with plantar-dorsi-flexion (positive ‘‘shift’’ test) as previously described (9). In addition, an experienced sports physician radiologically confirmed the clinical diagnosis. All CON participants were healthy and physically active asymptomatic individuals with no clinical history of chronic Achilles tendinopathy.

Exclusion criteria

All participants under the age of eighteen years, with a BMI greater than 30kg.m^{-2} and those with a history of current or past fluoroquinolone antibiotic use (within the last 12 months) were excluded from both previously published studies. In addition participants with a history of lower limb surgery, as well as, individuals (i) on chronic medications, (ii) diagnosed with connective tissue disorders or (iii) diseases that could be associated with a tendinopathy, such as, but not limited to, Ehlers-Danlos Syndrome, benign hypermobility joint syndrome, rheumatoid arthritis, systemic lupus erythematosus, hyperparathyroidism, renal insufficiency, diabetes mellitus and familial hypercholesterolemia, were excluded from this study.

Participants with a history of Achilles tendon rupture, insertional Achilles tendinopathy, lower limb deformities, lower limb injury or previous local corticosteroid injections in the Achilles tendon or the area surrounding the Achilles tendon prior to the onset of symptoms were excluded from this study.

Ethical considerations

Ethical clearance for this study was obtained from the Human Research Ethics Committee of the Faculty of Health Sciences at the University of Cape Town (HREC number 279/2016; Appendix A). This study was conducted according to the principles of the Declaration of Helsinki (123), International conference of Harmonisation (ICH), the South African Good Clinical Practice (GCP) and law. This study was covered by the no fault liability insurance policy of the University of Cape Town. Participants consented to partake in this study prior to participation and were informed that they were able to withdraw from it without prejudice. Their information was de-identified, de-personalised and kept in an access-controlled facility.

2.2.2 Participant visit

During the first visit to the UCT's Health through Physical Activity, Lifestyle and Sport (HPALS) Research Centre, located at the Sport Science Institute of South Africa (SSISA) in Cape Town, the participants in both previously published studies were asked to (i) give written informed consent (Appendix C), (ii) complete demographic and sporting details (which included the grading of injuries, refer to Appendix D, section H), lifestyle and habits history, as well as, personal and family medical history (specifically tendon, ligament and joint capsule injuries) questionnaires (Appendix D), (iii) donate 5 millilitres of antecubital venous blood, and (iv) complete a quantitative sensory testing. The sub-set of TEN participants who were recruited for the original Mkumbuzi study (24) were also asked to complete additional pain self-report questionnaires (CON = 74 and TEN = 81) whereby they additionally graded the pain which they experienced in their Achilles tendons, which was further grouped as severe (SEV) or non-severe (Table 2.1). Blood samples were taken by a qualified phlebotomist for both studies, while Grey scale and Doppler ultrasonography of both Achilles tendons were done on all participants included in the second published study (24) during a second visit.

Table 2.1 Grading system classifying the severity of pain for this study

Grade at injury	Description of pain	Severity
Grade 1	Pain only after exercise	Non-severe
Grade 2	Pain during exercise, but did not alter training	Non-severe
Grade 3	Pain during exercise, but causes you to alter your training	**SEVERE**
Grade 4	Pain which causes you to stop training	**SEVERE**

Pain self-report

The TEN participants previously recruited in the second published study (24) were asked to complete the Victorian Institute of Sports Assessment-Achilles Questionnaire (VISA-A) (Appendix E). The VISA-A is a validated and reliable self-administered eight-question questionnaire which evaluates the clinical severity of tendinopathy symptoms and their impact on physical activity (22). They rated their current pain on the short-form McGill pain questionnaire (sf-MPQ) (Appendix F) and completed the short-form Brief Pain Inventory (sf-BPI) scale (Appendix G). The sf-MPQ assessed the quality of subjective pain and its intensity, while the sf-BPI assessed the temporal behaviour of pain (22,24).

Grey scale ultrasonography of both Achilles tendons

Grey scale ultrasonography was performed with the participants lying in prone and their feet overhanging the edge of the examination table. The tendons were examined in longitudinal and axial planes from the osteotendinous junction to the musculotendinous junction. Three ultrasound criteria were examined: echotexture, intrasubstance tears and overall tendon thickness. Echotexture was evaluated by identifying areas of hypoechoic change within the tendon and scored semi-quantitatively from 0 (normal tendon) to 10 (diffuse hypoechoic areas). Intrasubstance tears were identified as present if there was a focal discontinuity of theechogenic band in the substance of the tendon, which was partially or completely thick. Tendonthickness was measured as the maximal antero-posterior diameter in millimetres (124).

2.3 DNA extraction and genotyping

Approximately 5 millilitres of venous blood was obtained by a trained phlebotomist using the antecubital forearm vein of each participant and then stored in an EDTA vacutainer tube at -20°C in an access-controlled freezer and laboratory prior to DNA extraction and genotyping using previously described methods (125), modified by Mokone (126) (Appendix H).

Standard and custom made TaqMan™ Genotyping Assays (Thermo Fischer, Waltham, MA, USA) were used to genotype the DNA samples, from the study by Mkumbuzi (24), for *VEGFA* rs699947 (-2578 C>A), *VEGFA* rs2010963 (-634 G>C), *KDR* rs2071559 (-604 G>A) and *KDR* rs1870377 (1719 T>A) polymorphisms according to the manufacturer's recommendations

(Figures 1.3 and 1.4). Some of the DNA samples (CON = 120 and TEN = 104) were previously genotyped for these four polymorphisms, including *VEGFA* rs1570360 (-1154 G>A), and the genotype data was published (50). Unfortunately, due technical problems with the TaqMan™ Genotyping Assays, we could not genotype the DNA samples for rs1570360 (-1154 G>A), previously recruited and included in Mkumbuzi (24). This polymorphism was therefore not included in this dissertation.

The *VEGFA* and *KDR* SNPs were selected according to their functional significance, previous associations of multi-factorial pain and affective phenotypes. Negative controls (no DNA) and five repeat samples of a known genotype were included on each polymerase chain reaction (PCR) plate as a quality control measure for reliable genotyping and detection of possible contamination. Genotypes were called using the Thermo Fisher Cloud suite (SDS3.2 software) (Figure 2.1 A to D) with an average call rate of 80% and confirmed by two independent investigators. Sanger sequencing (Figure 2.2 A to C) was used to confirm the genotypes for the *KDR* rs1870377 polymorphism due to the low A-allele frequency in the population (Frequency as taken from Ensemble: T-allele = 76.5% and A-allele = 23.6%). Sanger sequencing alternatively known as chain termination, sequences DNA according to the selective incorporation of chain-terminating dideoxy nucleotides by DNA polymerase which incorporates deoxynucleotide triphosphates or chain-terminating dideoxy nucleotide triphosphates (ddNTPs) during in-vitro DNA replication, which will determine the nucleotide sequence of the DNA. This process requires the annelation of DNA to an oligonucleotide primer and is then extended by DNA polymerase. Thereafter, rate-limiting concentrations of ddNTPs stop the elongation reactions and result in DNA fragments to be distinguishedaccording to various lengths. These lengths will be marked with letters, which will identify where the participant is homozygous or heterozygous (127). All laboratory work was performed at the Health for Physical Activity, Lifestyle and Sports (HPALS) Research Centreof the University of Cape Town.

2.4 Statistical analysis

The programming environment R 4.2.0 (R Development Core Team, 2023) and IBM SPSS Statistics (version 28.0.1.0) were used for data analysis. The TEN and CON participants' general characteristics, injury, sporting and genotype data from the previously published studies were combined for the results presented in Chapter 3 of this dissertation, while only the

data from the second published study was analysed and presented in Chapter 4 (24,50). The distribution of continuous data was analysed using the Shapiro-Wilk normality test and normally distributed data was presented as means standard deviations, while median and interquartile ranges (Q1, Q3) were used to present non-parametric data. Unpaired t-tests or one-way ANOVAs were used for continuous normally distributed data, while Mann Whitney-U or Kruskal-Wallis tests were used for non-parametric data. Categorical data were compared using the Chi-squared or Fisher's Exact tests and presented as percentages. Analysis of the participant characteristics revealed possible confounding variables which were adjusted accordingly, such as age, weight and BMI. Statistical significance was accepted at $p<0.05$.

The R package *genetics* (128) and *SNPassoc* (129) were used to analyse the differences in genotype and allele frequencies among the groups as well as Hardy-Weinberg equilibrium (HWE) for each SNP. Inferred haplotypes (*VEGFA:* C-G, C-C, A-G and A-C; *KDR*:G-T, G-A, A-T and A-A) were constructed for and as a proxy for gene-gene interaction between *VEGFA* and *KDR*. Allele-allele interactions (*VEGFA/KDR*: C-G, C-A, A-G and A-A) were constructed using the genotype data and R package *haplo.stats* (130).

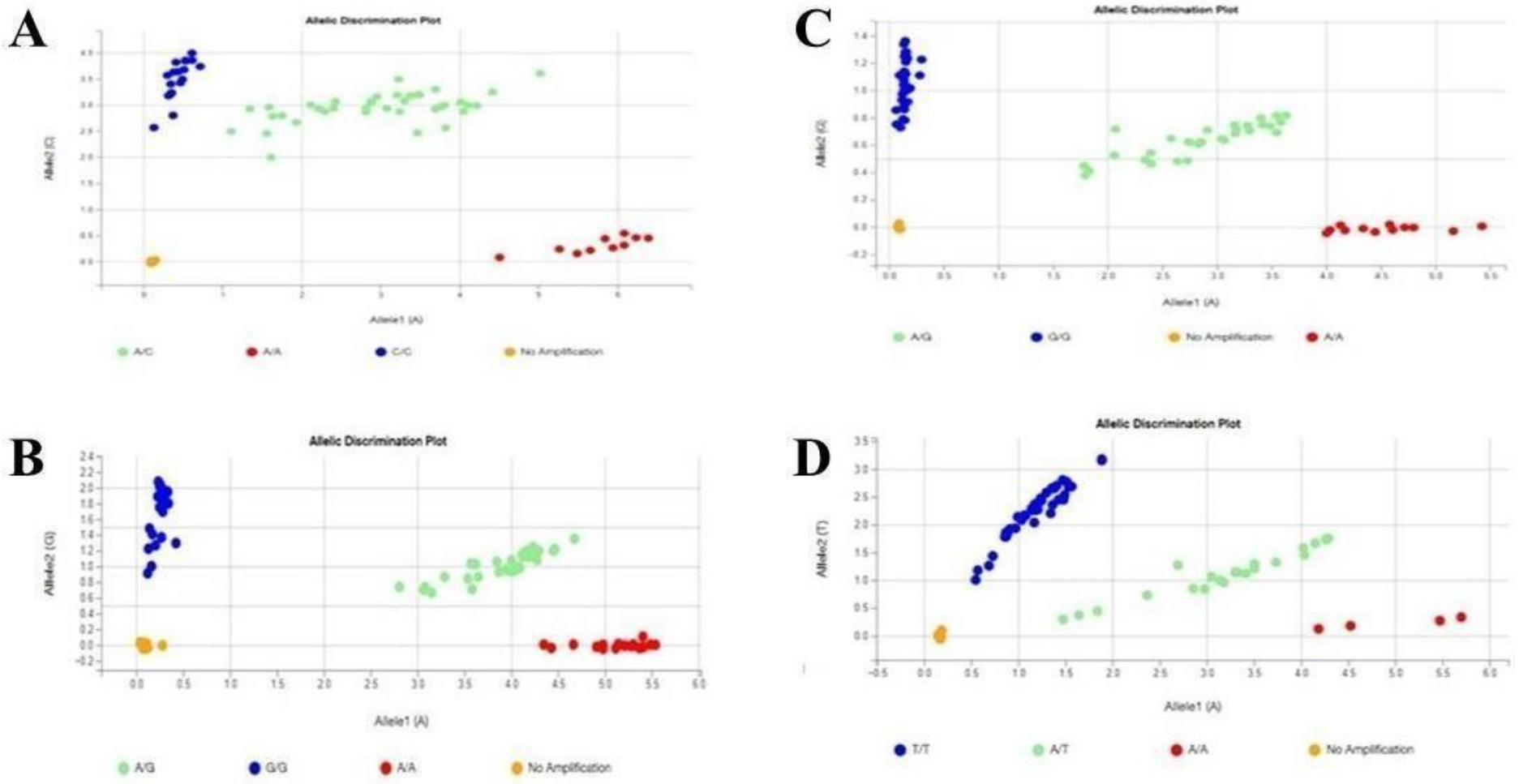

Figure 2.1: (A to D) Typical scatter of VIC and FAM labelled allele when preforming real-time fluorescence PCR allele discrimination: (A) *VEGFA* rs699947, (B) *VEGFA* rs2010963, (C) *KDR* rs2071559 and (D) *KDR* rs1870377. The graph plots the fluorescence of the FAM labelled allele versus the fluorescence of the VIC labelled allele. Genotypes are automatically called by the SDS3.2 software (Thermo Fisher, Waltham, MA, USA). Blue data points are homozygous for the FAM labelled allele, red data points are homozygous for the VIC labelled allele and the green data points are heterozygous for the FAM and VIC labelled alleles. The yellow data points indicate no amplification in the negative (water).

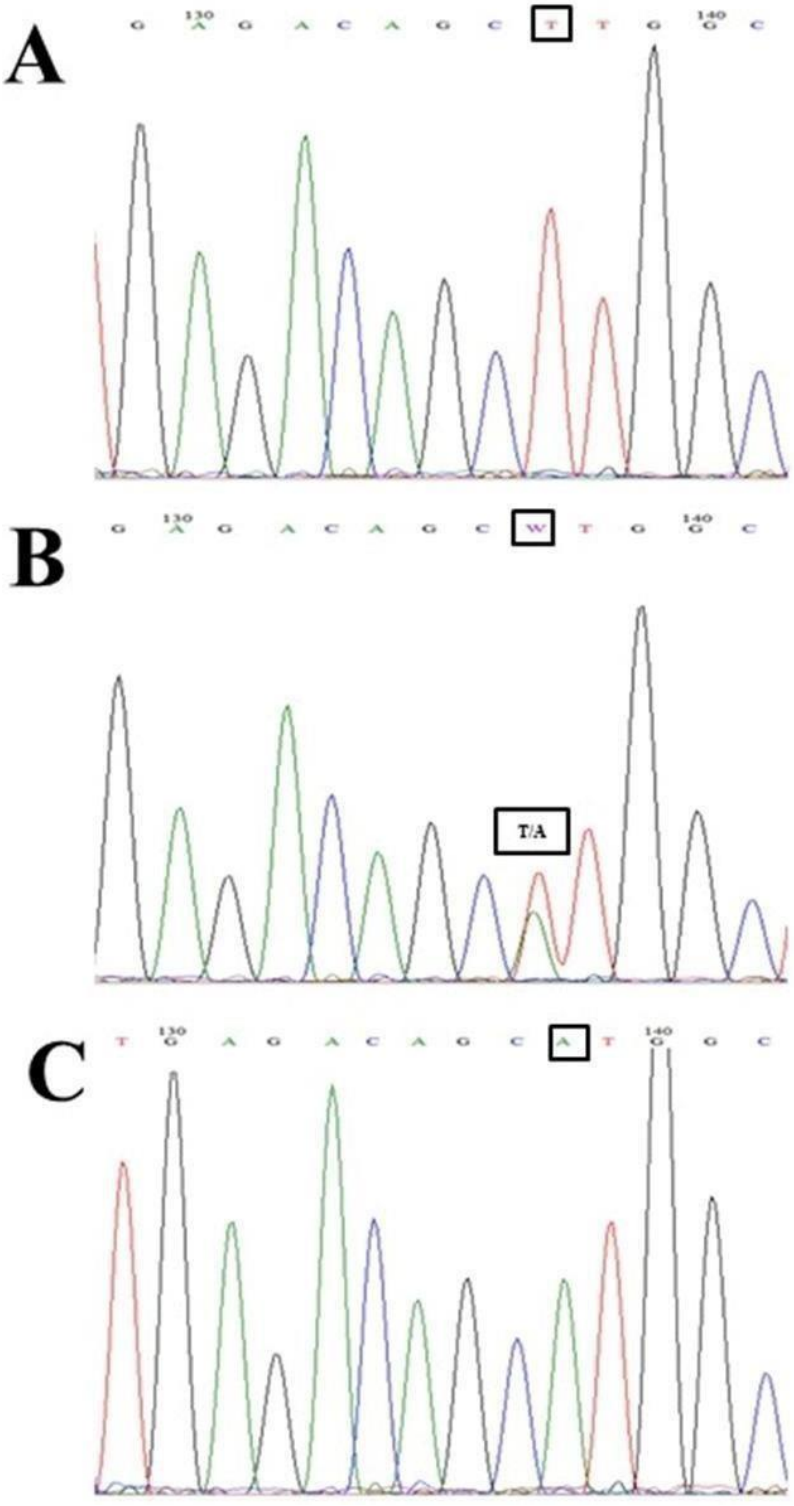

Figure 2.2: (A to C) The electropherogram images illustrating the Sanger sequencing for *KDR*rs1870377 (A) T/T; (B) T/A and (C) A/A genotypes. This participant was heterozygous as highlighted by the presence of both the T and A alleles indicated by a W. The black squares highlight the genotypes present in this individual.

Chapter 3

Association of the *VEGFA* and *KDR* Genes with Chronic Achilles Tendinopathy

3. 1 Participant characteristics

When the two previously published cohorts (24,50) were combined and re-analysed, the combined CON and TEN groups differed significantly in age, weight and BMI (Table 3.1). The combined CON (38.8 ± 12.1 years, n = 194) group was on average significantly younger (p = 0.010) at the time of recruitment compared to the combined TEN (42.2 ± 12.8 years, n = 185) group at the time of their reported first injury (Table 3.1). Although the CON (41.0 ± 14.1 years, n = 74) and TEN (42.3 ± 11.6 years, n = 81, p = 0.562) groups were matched for age in the second published cohort (Supplementary Table S3.1), the CON participants were significantly youngerin the first published cohort (CON: 37.3 ± 10.4 years vs TEN: 42.2 ± 13.9 years, p = 0.004) (Supplementary Table S3.2). The average age of the combined TEN group at the time of recruitment was 47.0 ± 12.0 years, which was on average 3.0 ± 5.0 years (ranging from 1 to 33 years) after their first reported injury. The combined, as well as the individual cohorts, CON and TEN groups were matched for sex and height (Table 3.1, Supplementary Tables S3.1 and S3.2). When covaried for age at the time of recruitment, the combined CON group, as well as only the first published cohort, were significantly lighter, with a corresponding lower BMI, at the time of recruitment compared to their respective TEN groups (combined CON: 71.7 ± 12.5 kg vs TEN: 77.0 ± 16.7 kg, p <0.001 and combined CON: 23.3 ± 3.0 $kg.m^{-2}$ vs TEN: 25.0 ± 4.1 $kg.m^{-2}$, p <0.001) (Table 3.1, Supplementary Tables S3.1 and S3.2).

One-hundred and sixty-three combined TEN participants reported the type of activity which resulted in their injury, of which 115 (70.6%) were injured while running.

3.2 *VEGFA* and *KDR* Genotype Associations on Participant Characteristics

There were significantly more (p = 0.036) males in the *VEGFA* rs699947 AA genotype group (74.4%, n = 67) compared to the CA (58.2%, n = 103) and CC (60.9%, n = 56) genotype groups (Supplementary Tables S3.3). When co-varied for sex, participants with the *VEGFA* rs699947 AA genotype were significantly heavier (77.8 ± 18.1 kg, n = 90, p = 0.049) than those with the CC (73.7 ± 13.3 kg, n = 92) or the CA (73.1 ± 13.8 kg, n = 177) genotypes (Supplementary Table S3.3). In addition, participants with the *VEGFA* rs699947 AA (177.7 ± 9.5 m, n = 87, p = 0.003) genotype were significantly taller at the time of injury compared to the CC (175.1 ± 9.1 m, n = 79) or CA (173.7 ± 8.7, n = 164) genotypes. The *VEGFA* rs699947 genotype groups were matched for age and BMI (Supplementary Tables S3.3). The *VEGFA* rs2010963, *KDR* rs1870377 and *KDR* rs2071559 genotype groups were matched to age, sex, height, weight and BMI (Supplementary Tables S3.4 to S3.6).

Table 3.1: Participant characteristics of the combined previously published cohorts' (24,50) asymptomatic control (CON) and chronic Achilles Tendinopathy (TEN) groups

	CON (n = 194)	**TEN** (n = 185)	**P-value**
Age (years) [1]	38.8 ± 12.1	42.2 ± 12.8	**0.010**
Sex (% male)	63.8	66.4	0.593
Height (cm)	174.9 ± 9.5	175.2 ± 8.6	0.702
Weight (kg) [2]	71.7 ± 12.5	77.0 ± 16.7	**0.001 (0.001)** [3]
BMI ($kg.m^{-2}$) [2]	23.3 ± 3.0	25.0 ± 4.1	**<0.001 (0.001)** [3]

Except for sex, which is expressed as a percentage, values are expressed as mean ± standard deviation

P-values in bold typeset indicates significance

BMI, body mass index; cm, centimetres; kg, kilograms; m, metres

[1]Age for the CON and TEN groups are at the time of recruitment and first injury, respectively

[2]Weight and therefore BMI are self-reported values at the time of recruitment for both groups

[3]Adjusted p-values for age at recruitment

Except for the *KDR* rs2071559 TEN (p = 0.037), combined CON and combined TEN groups (p = 0.047), the *VEGFA* rs699947 and rs2010963, as well as the *KDR* rs1870377 genotypes were in Hardy-Weinberg Equilibrium (HWE) in all groups (Supplementary Tables S3.7).

3.3 Genotype and Allele Associations on Achilles Tendinopathy

3.3.1 *VEGFA* rs699947 (-2578 C>A)

There were no significant differences in the *VEGFA* rs699947 genotype (p = 0.129) or allele (p = 0.205) frequency distributions between the combined CON and TEN groups. Similarly, there were also no significant differences in the genotype frequency distributions between these groups during a sub-analysis of the male (p = 0.154) and female (p = 0.547) participants from the combined groups (Figure 3.1). The genotype and allele frequencies were similar to the published values for the European population in the Ensemble public database (www.Ensemble.org, accessed during August 2023) (Table 3.2).

Table 3.2: Genotype and allele frequency distribution of the *VEGFA* rs699947 (-2578 C>A) polymorphism in the combined asymptomatic control (CON) and chronic Achilles Tendinopathy (TEN) groups of the previously published cohort (50) and the cohort genotyped in this study, as well as the distribution within the European population (EUR) in the Ensemble public database (www.Ensemble.org).

Genotype or Allele	**CON** (N = 183)	**TEN** (N = 176)	**P-value**	**EUR** (N = 503)
CC	30.1 (55)	21.0 (37)		27.2 (137)
CA	45.4 (83)	53.4 (94)	0.129	46.5 (234)
AA	24.6 (45)	25.6 (45)		26.2 (132)
A	47.3 (173)	52.3 (184)	0.205	49.5 (498)

Genotype and minor allele frequencies are represented as frequencies with the number (n) of participants in parentheses

The total number (N) of participants genotyped in each group (CON, TEN and EUR) is also indicated

VEGFA, Vascular endothelial growth factor A

The Ensemble database was accessed during August 2023

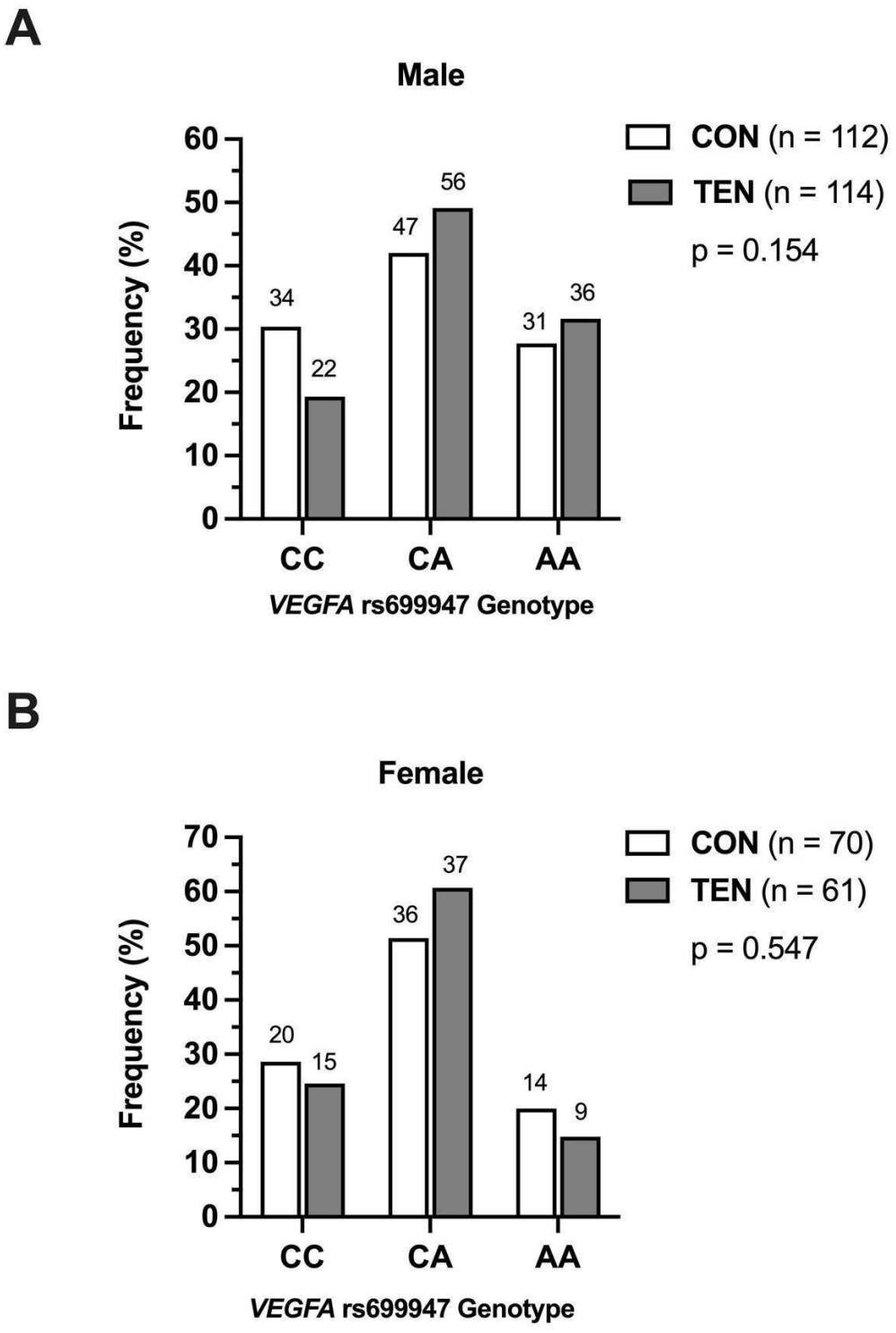

Figure 3.1: Genotype frequency distributions of the *VEGFA* rs699947 (-2578 C>A) polymorphism of the combined CON (white bars) and combined TEN (grey bars) groups in the sub-analysis of **(A)** male and **(B)** female participants. The p-values and number of participants (n) in each group are indicated on the graphs. The number of participants in each genotype group is also indicated above the bars.

3.3.3 *VEGFA* rs2010963 (-634 G>C)

There were no significant differences in the *VEGFA* rs2010963 genotype (p = 0.342) or allele (p = 0.281) frequency distributions between the CON and TEN groups (Table 3.3). Similarly, there were also no significant differences in the genotype frequency distributions between the CON and TEN groups when only male participants were compared (p = 0.445) or when only female (p = 0.183) participants were compared (Figure 3.2). The genotype and allele frequencies were similar to the published values for the European population in the Ensemble public database (www.Ensemble.org, accessed during August 2023) (Table 3.3).

Table 3.3: Genotype and allele frequency distribution of the *VEGFA* rs2010963 (-634 G>C) polymorphism in the combined asymptomatic control (CON) and chronic Achilles Tendinopathy (TEN) groups of the previously published cohort (50) and the cohort genotyped in this study, as well as the distribution within the European population (EUR) in the Ensemble public database (www.Ensemble.org).

Genotype or Allele	**CON** (N = 188)	**TEN** (N = 180)	**P-value**	**EUR** (N = 503)
GG	42.0 (79)	45.0 (81)		47.5 (239)
GC	43.6 (82)	45.6 (82)	0.342	43.3 (218)
CC	14.4 (27)	9.4 (17)		9.1 (46)
C	36.2 (136)	32.1 (106)	0.281	30.8 (310)

Genotype and minor allele frequencies are represented as frequencies with the number (n) of participants in parentheses

The total number (N) of participants genotyped in each group (CON, TEN and EUR) is also indicated

VEGFA, Vascular endothelial growth factor A

The Ensemble database was accessed during August 2023

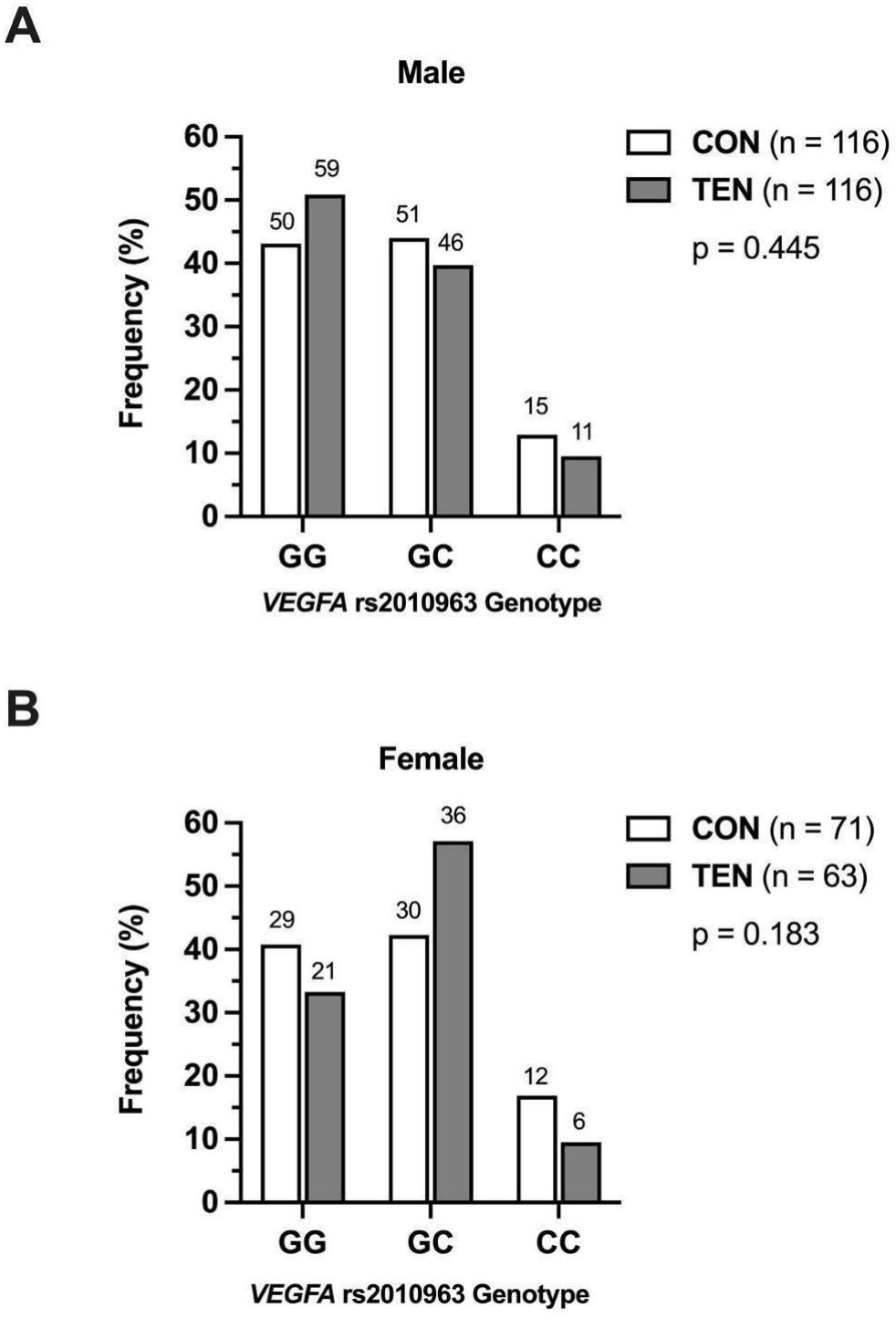

Figure 3.2: Genotype frequency distributions of the *VEGFA* rs2010963 (-634 G>C) polymorphism of the combined CON (white bars) and combined TEN (grey bars) groups in the sub-analysis of **(A)** male and **(B)** female participants. The p-values and number of participants (n) in each group are indicated on the graphs. The number of participants in each genotype group is also indicated above the bars.

3.3.4 *KDR* rs2071559 (-604 G>A)

There were no significant differences in the *KDR* rs2071559 genotype (p = 0.094) or allele (p = 0.060) frequency distributions between the combined CON and TEN groups (Table 3.4). Similarly, there were no significant differences in the genotype frequency distributions between the CON and TEN groups during a sub-analysis of the male (p = 0.475) and female (p = 0.196) participants (Figure 3.3). The genotype and allele frequencies were similar to the published values for the European population in the Ensemble public database (www.Ensemble.org, accessed during August 2023) (Table 3.4).

Table 3.4: Genotype and allele frequency distribution of the *KDR* rs2071559 (-604 G>A) polymorphism in the combined asymptomatic control (CON) and chronic Achilles Tendinopathy (TEN) groups of the previously published cohort (50) and the cohort genotyped in this study, as well as the distribution within the European population (EUR) in the Ensemble public database (www.Ensemble.org).

Genotype or Allele	**CON** (N = 189)	**TEN** (N = 181)	**P-value**	**EUR** (N = 503)
GG	32.8 (62)	28.2 (51)		22.7 (114)
GA	47.1 (89)	42.0 (76)	0.094	51.7 (260)
AA	20.1 (38)	29.8 (54)		25.6 (129)
A	43.7 (165)	50.8 (184)	0.060	51.5 (518)

Genotype and minor allele frequencies are represented as frequencies with the number (n) of participants in parentheses

The total number (N) of participants genotyped in each group (CON, TEN and EUR) is also indicated

KDR, Kinase Domain Receptor

The Ensemble database was accessed during August 2023

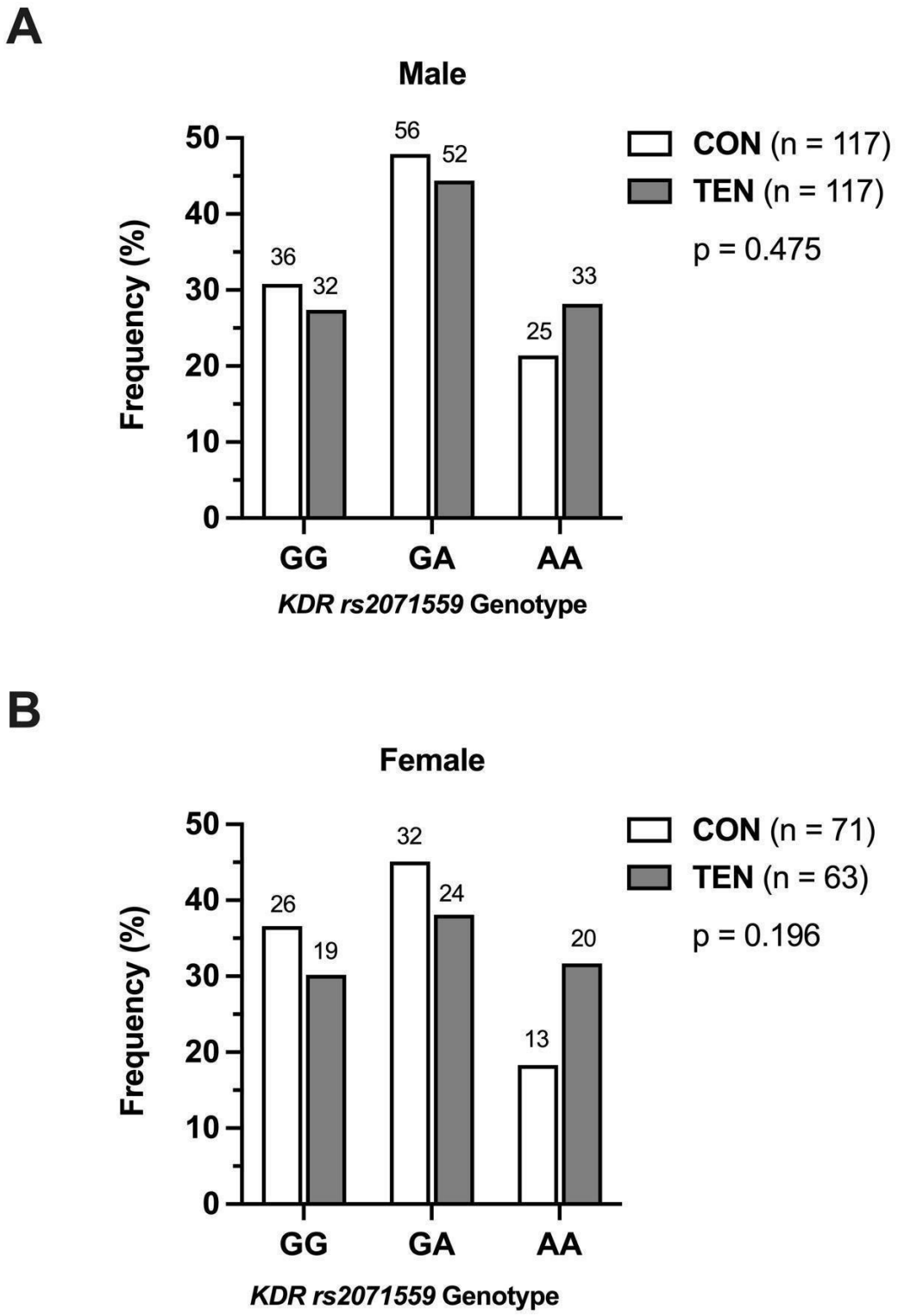

Figure 3.3: Genotype frequency distributions of the *KDR* rs2071559 (-604 G>A) polymorphism of the combined CON (white bars) and the combined TEN (grey bars) groups in the sub-analysis of **(A)** male and **(B)** female participants. The p-values and number of participants (n) in each group are indicated on the graphs. The number of participants in each genotype group is also indicated above the bars.

3.3.5 *KDR* rs1870377 (1719 T>A)

Finally, there were no significant differences in the *KDR* rs1870377 genotype (p = 0.741) or allele (p = 0.603) frequency distributions between the CON and TEN groups (Table 3.5). Similarly, there were no significant differences in the genotype frequency distributions between the CON and TEN groups when only male (p = 0.833) and only female (p = 0.554) participants were evaluated (Figure 3.4). The genotype and allele frequencies were similar to the published values for the European population in the Ensemble public database (www.Ensemble.org, accessed during August 2023) (Table 3.5).

Table 3.5: Genotype and allele frequency distribution of the *KDR* rs1870377 (1719 T>A) polymorphism in the combined asymptomatic control (CON) and chronic Achilles Tendinopathy (TEN) groups of the previously published cohort (24,50) and the cohort genotyped in this study, as well as the distribution within the European population (EUR) in the Ensemble public database (www.Ensemble.org).

Genotype or Allele	**CON** (N = 182)	**TEN** (N = 174)	**P-value**	**EUR** (N = 503)
TT	57.1 (104)	55.2 (96)		58.1 (292)
TA	37.4 (68)	37.4 (65)	0.741	37.0 (186)
AA	5.5 (10)	7.5 (13)		5.0 (25)
A	24.2 (88)	55.2 (96)	0.603	23.5 (236)

Genotype and minor allele frequencies are represented as frequencies with the numbers from each cohort in parentheses

The total number of participants genotyped in each group (CON, TEN and EUR) is also indicated

KDR, Kinase Domain Receptor

The Ensemble database was accessed during August 2023

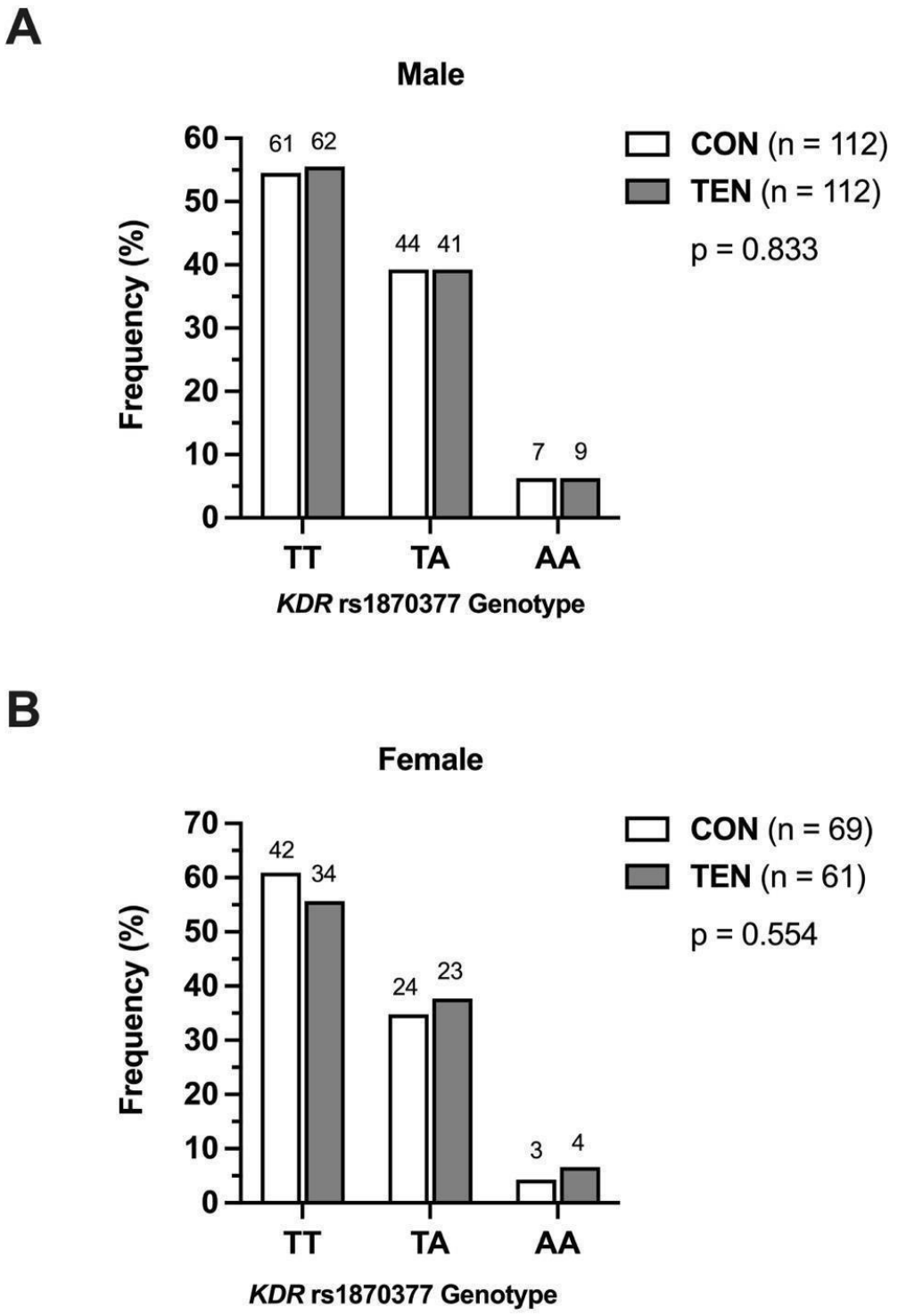

Figure 3.4: Genotype frequency distributions of the *KDR* rs1870377 (1719 T>A) polymorphism of the combined CON (white bars) and the combined TEN (grey bars) groups in the sub-analysis of **(A)** male and **(B)** female participants. The p-values for the male (TT vs TA vs AA) and female (TT vs TA and AA), as well as the number of participants (n) in each group are indicated on the graphs. The number of participants in each genotype group is also indicated above the bars.

3.4 *VEGFA* and/or *KDR* Haplotype Associations on Achilles Tendinopathy

Inferred haplotypes were constructed for the two *VEGFA* (rs699947 C>A and rs2010963 G>C) and the two *KDR* (rs2071559 G>A and rs1870377 T>A) polymorphisms. No significant differences were observed in the *VEGFA* (additive model p = 0.531) and *KDR* (additive model p = 0.271) haplotype frequency distributions between the combined CON and TEN groups (Figure 3.5).

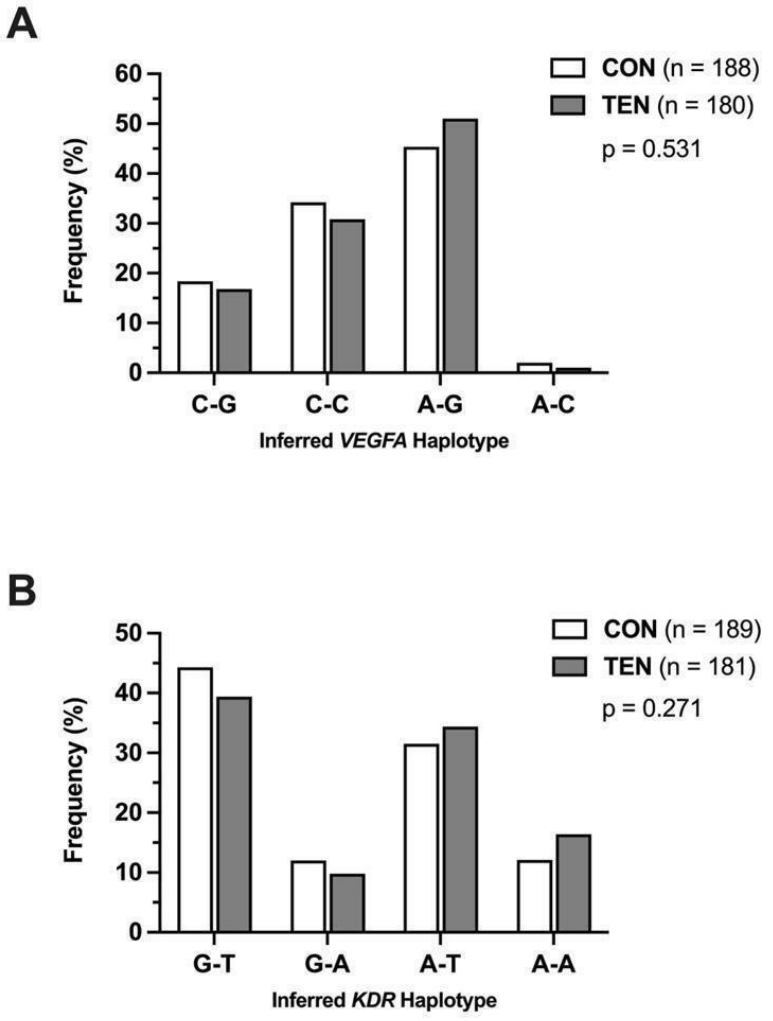

Figure 3.5: The relative frequencies of the inferred haplotypes constructed from **(A)** *VEGFA* (rs699947 C>A and rs2010963 G>C) and **(B)** *KDR* (rs2071559 G>A and rs1870377 T>A) polymorphisms in the CON (white bars) and the TEN (grey bars) for all participants in the combined cohorts. The global p-values (additive model of inheritance) and the number of participants (n) in each group is indicated on the graphs.

Similarly, there were no significant differences in the *VEGFA* and *KDR* inferred haplotype frequency distributions between the CON and TEN groups when only the male (additive model *VEGFA:* p = 0.468 and additive model *KDR:* p = 0.378) and female (*VEGFA:* p = 0.987 and *KDR:* p = 0.352) participants were analysed (Figure 3.6).

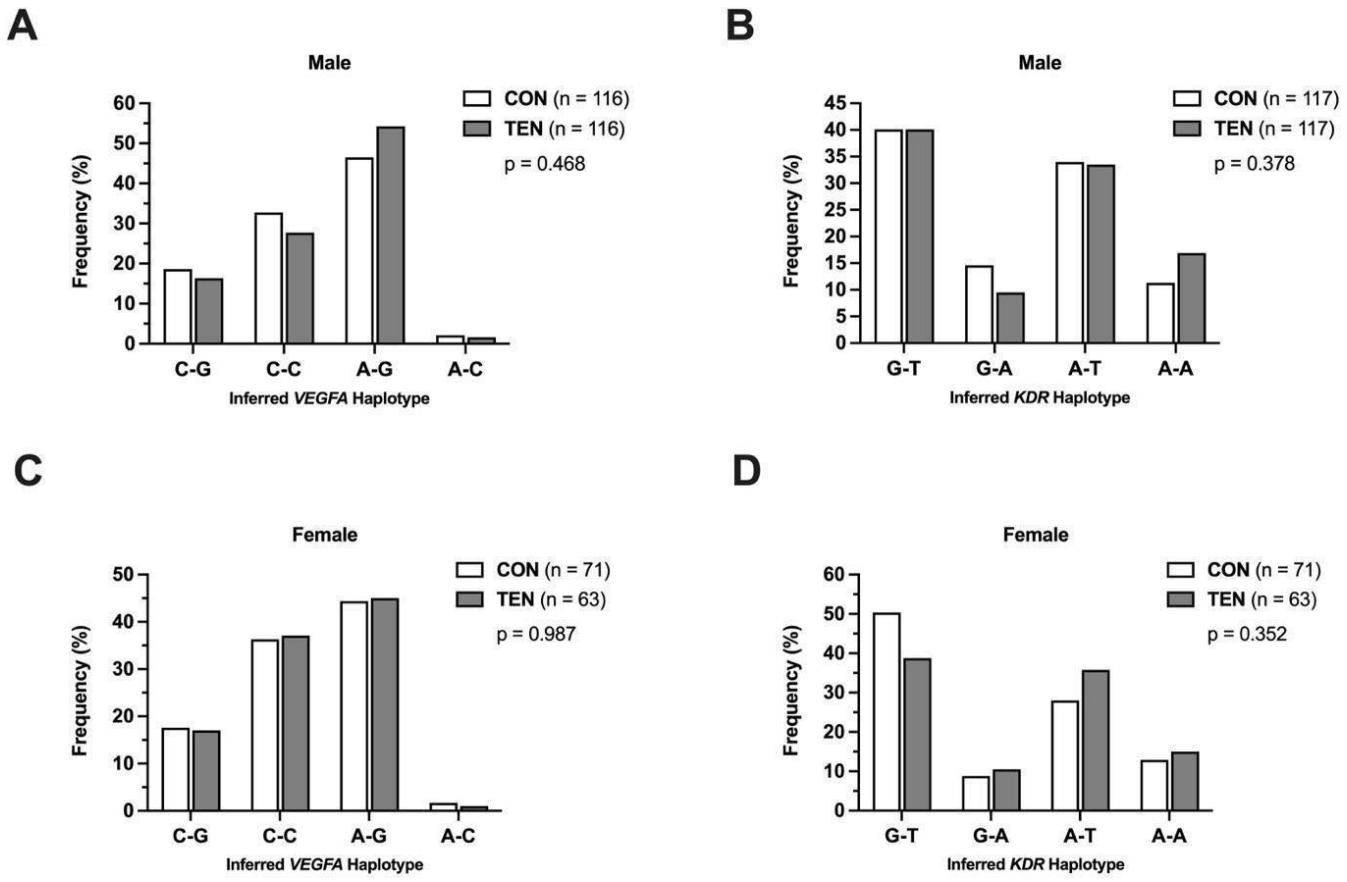

Figure 3.6: The relative frequencies of the inferred haplotypes constructed for (*VEGFA* rs699947 C>A and rs2010963 G>C) **(A)** male and **(C)** female participants; and for *KDR* (rs2071559 G>A and rs1870377 T>A) **(B)** male and **(D)** female participants in the CON (white bars) and TEN (grey bars) groups in the combined cohorts. The global additive p-values and the number of participants (n) in each group is indicated on the graphs.

The *VEGFA* rs699947 (C>A) and *KDR* rs2071559 (G>A) allele-allele interactions were investigated because of their independent genotype distributions (24,50). Although, there were no significant differences (recessive model global p = 0.054) in the relative distribution of the *VEGFA* allele and *KDR* allele interactions (Figure 3.7), the C-G allele-allele interaction was overrepresented (p = 0.015) in the CON (29.6%) group compared to the TEN group (22.6%). There were no significant differences in the relative distribution of the *VEGFA* rs699947 and *KDR* rs2071559 allele-allele interactions when the male (recessive model p = 0.208) and female (recessive model p = 0.283) participants were analysed separately (Figure 3.8).

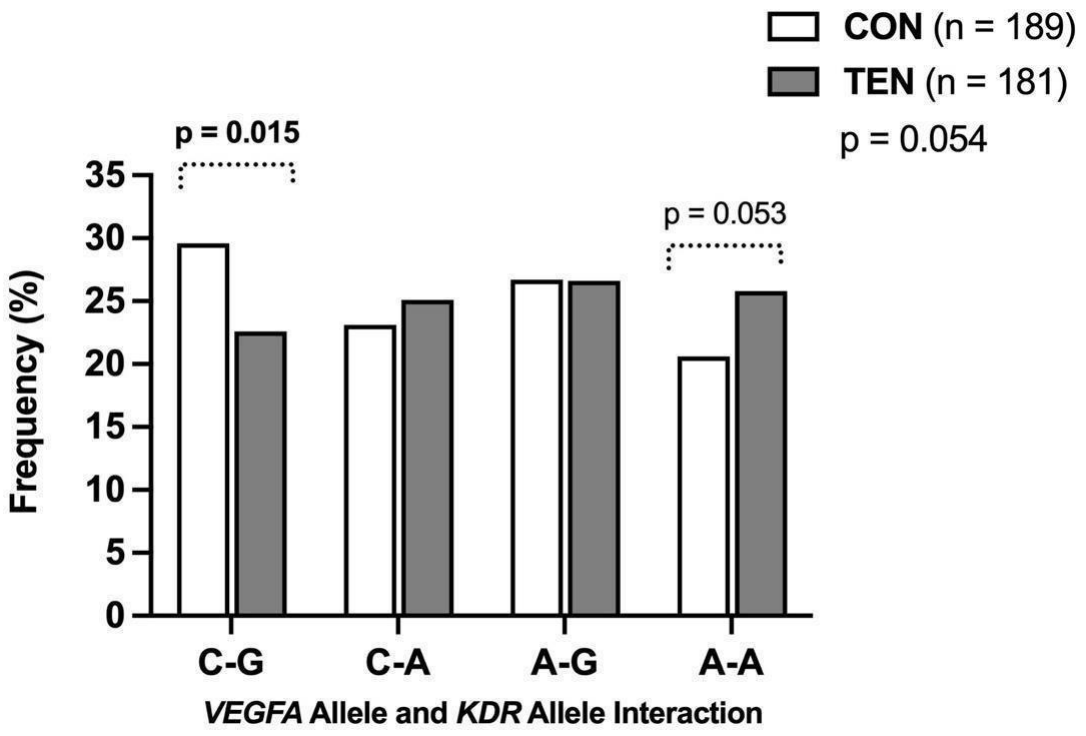

Figure 3.7: The relative frequencies of the *VEGFA* (rs699947 C>A) and *KDR* (rs2071559 G>A) allele-allele interactions in the CON (white bars) and TEN (grey bars) groups for all participants in the combined cohorts. The global p-values (recessive model of inheritance), the specific p-values for the C-G and A-A interactions, as well as the number of participants (n) in each group is indicated on the graphs.

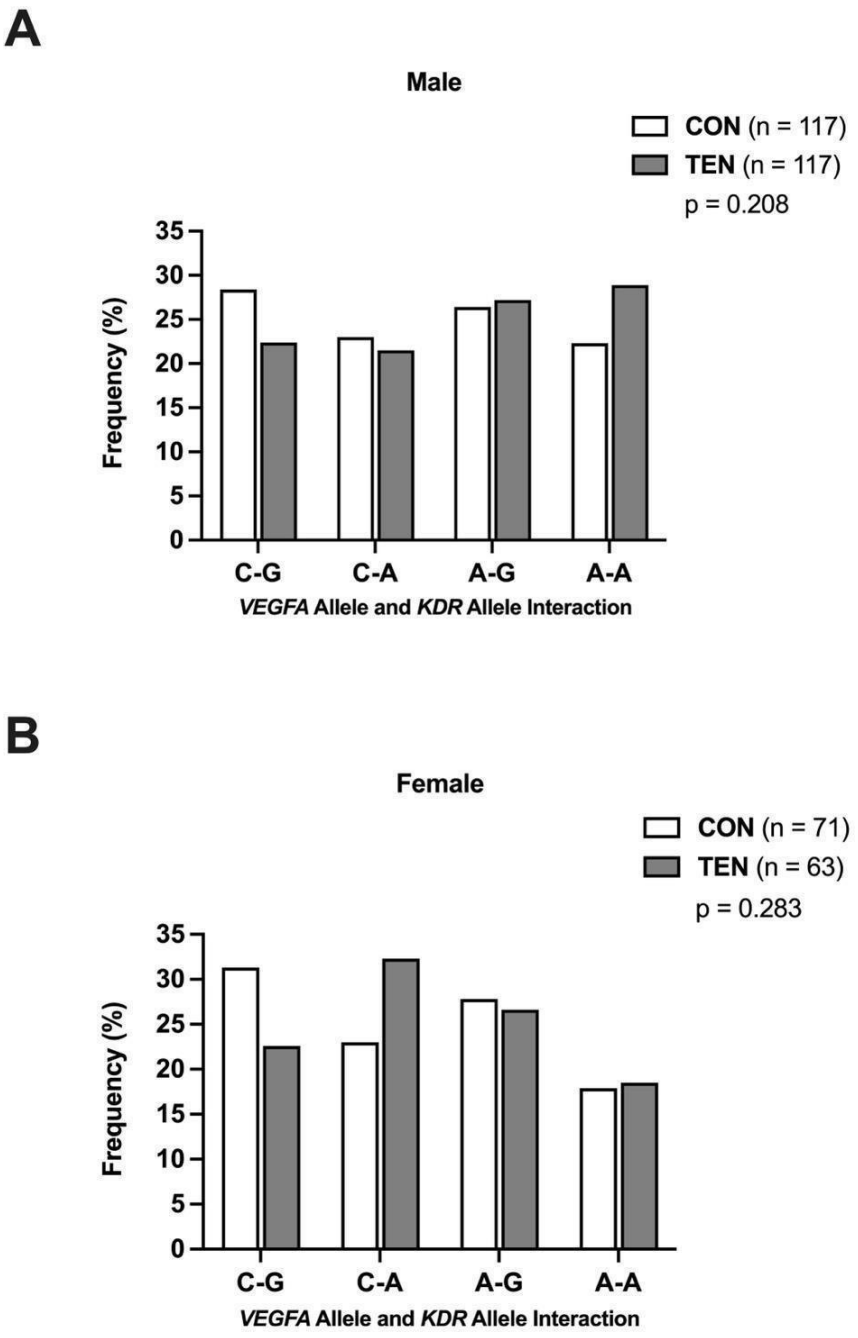

Figure 3.8: The relative frequencies of the **(A)** male and **(B)** female *VEGFA* (rs699947 C>A) and *KDR* (rs2071559 G>A) allele-allele interactions in the CON (white bars) and TEN (grey bars) for all participants in the combined cohorts. The global p-values (recessive model of inheritance) and the number of participants (n) in each group is indicated on the graphs.

3.5 *VEGFA* and *KDR* Genotype and Allele Associations on the Region and Severity of Injury

Of the 154 participants who reported the effect of pain on exercise at time of injury, 12.3% (n = 19), 23.4% (n = 36), 26.6% (n = 41) and 37.7% (n = 58) reported pain only after exercise (grade I), pain during exercise which does not alter exercise (grade II), pain during exercise which altered exercise (grade III) and pain that stopped exercise (grade IV) respectively. Participants who reported either grades III or IV pain at the time of injury (n = 99) were combined and analysed as a sub-group (SEV) (Table 2.1).

Of the 135 participants who reported the region of injury, 57.0% (n = 77) reported an injury to the middle-third of the tendon, 33.3% (n = 45) reported the lower-third and 9.6% (n = 13) reported injuring the upper-third or more than one region of the tendon. The 77 participants who reported an injury to the middle-third of the tendon (MID) were also analysed as a sub- group.

3.5.1 *VEGFA* rs699947 (-2578 C>A)

There were no significant differences in the *VEGFA* rs699947 genotype (p = 0.170) or allele (p = 0.322) frequency distributions between the CON group and the SEV sub-group that reported either a grade III or grade IV injuries (Table 3.6). Although not included in the analysis because of small sample sizes (n = 53), the CC (20.8%, n = 11), CA (49.1%, n = 26) and AA (30.2%, n = 16) genotype distributions of the TEN participants who reported grade I or grade II injuries were similar to the SEV sub-group.

Furthermore, there were no significant differences in the genotype (p = 0.694) or allele (p = 0.629) frequency distributions between the CON group and the TEN sub-group of participants who reported an injury to the middle-third of their Achilles tendon (MID) (Table 3.6). Interestingly, although not included in the analysis because of a small sample size (n = 45), the CC (13.3%, n = 6), CA (53.3%, n = 24) and AA (33.3%, n = 15) genotype distributions of the TEN participants who reported injury to lower-third of the Achilles tendon were different to the MID sub-group. In particular the CC genotype was lower in this TEN sub-group.

Table 3.6: Genotype and allele frequency distributions of the *VEGFA* rs699947 (-2578 C>A) polymorphism in the combined asymptomatic control (CON) groups, as well as the sub-groups of participants diagnosed with chronic Achilles tendinopathy that reported either a grade III or grade IV injury (SEV) or injury to the middle-third of the tendon (MID), from the previously published cohort (50) and the cohort genotyped in this study.

Genotype or Allele	**CON** (N = 183)	**SEV** (N = 98)	**P-value**	**MID** (N = 76)	**P-value**
CC	30.1 (55)	20.4 (20)		25.0 (19)	
CA	45.4 (83)	55.1 (54)	0.170	50.0 (38)	0.694
AA	24.6 (45)	24.5 (24)		25.0 (19)	
A	47.3 (173)	52.0 (102)	0.322	50.0 (76)	0.629

Genotype and minor allele frequencies are represented as frequencies with the numbers (n) in parentheses
The number of participants (N) genotyped in each group is indicated
VEGFA, Vascular endothelial growth factor A

3.5.2 *VEGFA* rs2010963 (-634 G>C)

There were no significant differences in the *VEGFA* rs2010963 genotype (p = 0.875) or allele (p = 0.705) frequency distributions between the CON and SEV groups (Table 3.7). Although not included in the analysis because of small sample sizes (n = 55), the GG (47.3%, n = 26), GC (45.5%, n = 25) and CC (7.3%, n = 4) genotype distributions of the TEN participants who reported grade I or grade II injuries were similar to the SEV sub-group.

There were also no significant differences in the genotype (p = 0.317) or allele (p = 0.483) frequency distributions between the CON and MID groups (Table 3.7). Although not included in the analysis because of a small sample size (n = 45), the GG (48.9%, n = 22), GC (40.0%, n = 18) and CC (11.1%, n = 5) genotype distributions of the TEN participants who reported injury to the lower-third of the Achilles tendon were similar to the MID sub-group.

Table 3.7: Genotype and allele frequency distribution of the *VEGFA* rs2010963 (-634 G>C) polymorphism in the combined asymptomatic control (CON) groups, as well as the sub-groups of participants diagnosed with chronic Achilles tendinopathy that reported either a grade III or grade IV injury (SEV) or injury to the middle-third of the tendon (MID), from the previously published cohort ((50) and the cohort genotyped in this study.

Genotype or Allele	**CON** (N = 188)	**SEV** (N = 98)	**P-value**	**MID** (N = 77)	**P-value**
GG	42.0 (79)	43.9 (43)		42.9 (33)	
GC	43.6 (82)	43.9 (43)	0.875	49.4 (38)	0.317
CC	14.4 (27)	12.2 (12)		7.8 (6)	
C	36.2 (136)	34.2 (67)	0.705	32.5 (50)	0.483

Genotype and minor allele frequencies are represented as frequencies with the numbers (n) in parenthesesThe number of participants (N) genotyped in each group is indicated

VEGFA, Vascular endothelial growth factor A

3.5.3 *KDR* rs2071559 (-604 G>A)

There were no significant differences in the *KDR* rs2071559 genotype (p = 0.146) or allele (p = 0.159) frequency distributions between the CON and SEV groups (Table 3.8). Although not included in the analysis because of small sample sizes (n = 55), the GG (23.6%, n = 13), GA (41.8%, n = 23) and AA (34.5%, n = 19) genotype distributions of the TEN participants who reported grade I or grade II injuries was similar to the SEV sub-group.

There were also no significant differences in the genotype (p = 0.097) or allele (p = 0.084) frequency distributions between the CON and MID groups (Table 3.8). Although not included in the analysis because of small sample sizes (n = 45), the GG (20.0%, n = 9), GA (48.9%, n =

22) and AA (31.1%, n = 14) genotype distributions of the participants who reported injury to lower-third of the Achilles tendon were similar to the MID sub-group.

Table 3.8: Genotype and allele frequency distribution of the *KDR* rs2071559 (-604 G>A) polymorphism in the combined asymptomatic control (CON) groups, as well as the sub-groups of participants diagnosed with chronic Achilles tendinopathy that reported either a grade III or grade IV injury (SEV) or injury to the middle-third of the tendon (MID), from the previously published cohort (50) and the cohort genotyped in this study.

Genotype or Allele	**CON** (N = 189)	**SEV** (N = 99)	**P-value**	**MID** (N = 77)	**P-value**
GG	32.8 (62)	30.3 (30)		28.6 (22)	
GA	47.1 (89)	39.4 (39)	0.146	39.0 (30)	0.097
AA	20.1 (38)	30.3 (30)		32.5 (25)	
A	43.7 (165)	50.0 (99)	0.159	51.9 (80)	0.084

Genotype and minor allele frequencies are represented as frequencies with the numbers (n) in parentheses
The number of participants (N) genotyped in each group is indicated
KDR, Kinase Domain Receptor

3.5.4 *KDR* rs1870377 (1719 T>A)

There were no significant differences in the *KDR* rs1870377 genotype (p = 0.489) or allele (p = 0.517) frequency distributions between the CON and SEV groups (Table 3.9). Although not included in the analysis because of small sample sizes (n = 52), the TT (55.8%, n = 29), TA (38.5%, n = 20) and AA (5.8%, n = 3) genotype distributions of the TEN participants who reported grade I or grade II injuries were similar to the SEV sub-group.

Furthermore, there were no significant differences in the genotype (p = 0.523) or allele (p = 0.432) frequency distributions between the CON and MID groups of this polymorphism. Although not included in the analysis because of small sample sizes (n = 44), the TT (63.3%, n = 28), TA (31.8%, n = 14) and AA (4.5%, n = 2) genotype distributions of the participants who reported injury to lower-third of the Achilles tendon were similar to the MID sub-group.

Table 3.9: *KDR* rs1870377 (1719 T>A) polymorphism in the combined asymptomatic control (CON) group, as well as the sub-groups of participants diagnosed with chronic Achilles tendinopathy that reported either a grade III or grade IV injury (SEV) or injury to the middle-third of the tendon (MID), from the previously published cohort (50) and the cohort genotyped in this study.

Genotype or Allele	**CON** (N = 182)	**SEV** (N = 96)	**P-value**	**MID** (N = 74)	**P-value**
TT	57.1 (104)	55.2 (53)		51.4 (35)	
TA	37.4 (68)	35.4 (34)	0.489	41.9 (31)	0.523
AA	5.5 (10)	9.4 (9)		6.8 (5)	
A	24.2 (88)	26.0 (52)	0.517	27.7 (41)	0.432

Genotype and minor allele frequencies are represented as frequencies with the numbers (n) in parentheses

The number of participants (n) genotyped in each group is indicated

KDR, Kinase Domain Receptor

3.6 *VEGFA* and *KDR* Haplotype and Allele Interaction Associations on the Region and Severity and region of Injury

Inferred haplotypes were constructed for the two *VEGFA* (rs699947 C>A and rs2010963 G>C) and the two *KDR* (rs2071559 G>A and rs1870377 T>A) polymorphisms. There were no significant differences in the relative frequencies of the inferred haplotypes constructed from the *VEGFA* polymorphisms between the CON and SEV (additive model p = 0.405, Figure 3.9A) or between the CON and MID (additive model p = 0.875, Figure 3.9B) groups. Similarly, there were also no significant differences between the relative frequencies of inferred haplotypes constructed from the *KDR* polymorphisms between the CON and SEV (additive model p = 0.339, Figure 3.9C) or between the CON and MID (additive model p = 0.314, Figure 3.9D) groups.

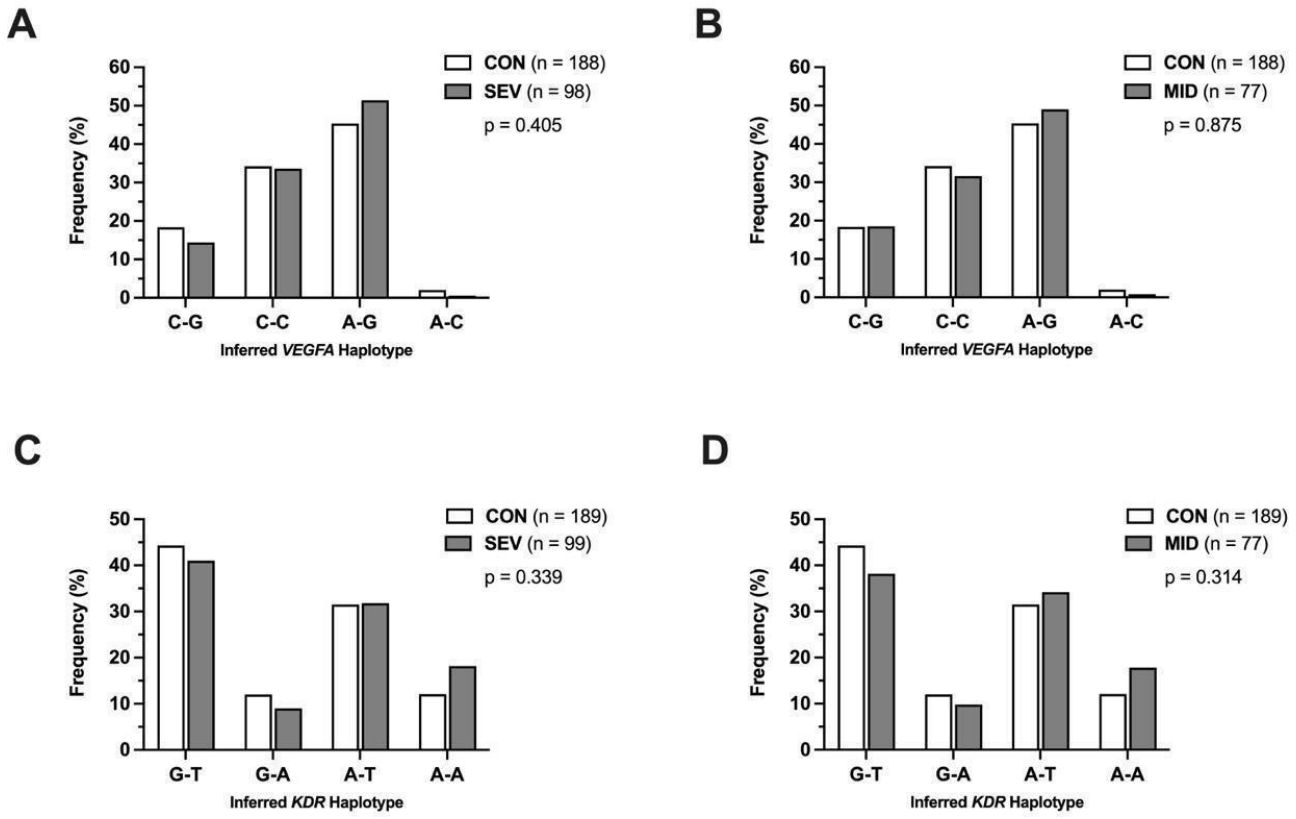

Figure 3.9: The relative frequencies of the inferred haplotypes constructed from **(A and B)** the two *VEGFA* (rs699947 C>A and rs2010963 G>C) and **(C and D)** the two *KDR* (rs2071559 G>A and rs1870377 T>A) polymorphisms in the CON (white bars) group, as well as the sub-groups of participants diagnosed with chronic Achilles tendinopathy that reported either **(A and C)** a grade III or grade IV injury (SEV) (grey bars) or **(B and D)** injury to the middle-third of the tendon (MID) (grey bars). The global p-values (additive model of inheritance)and the number of participants (n) in each group is indicated on the graphs.

There were no significant differences (recessive model p = 0.166) in the relative distribution of the *VEGFA* rs699947 and *KDR* rs2071559 allele-allele interactions between the CON group and SEV sub-group (Figure 3.10A). Similarly, there were also no significant differences (recessive model p = 0.226) in the relative distribution of the allele interactions between the CON group and MID sub-group (Figure 3.10B).

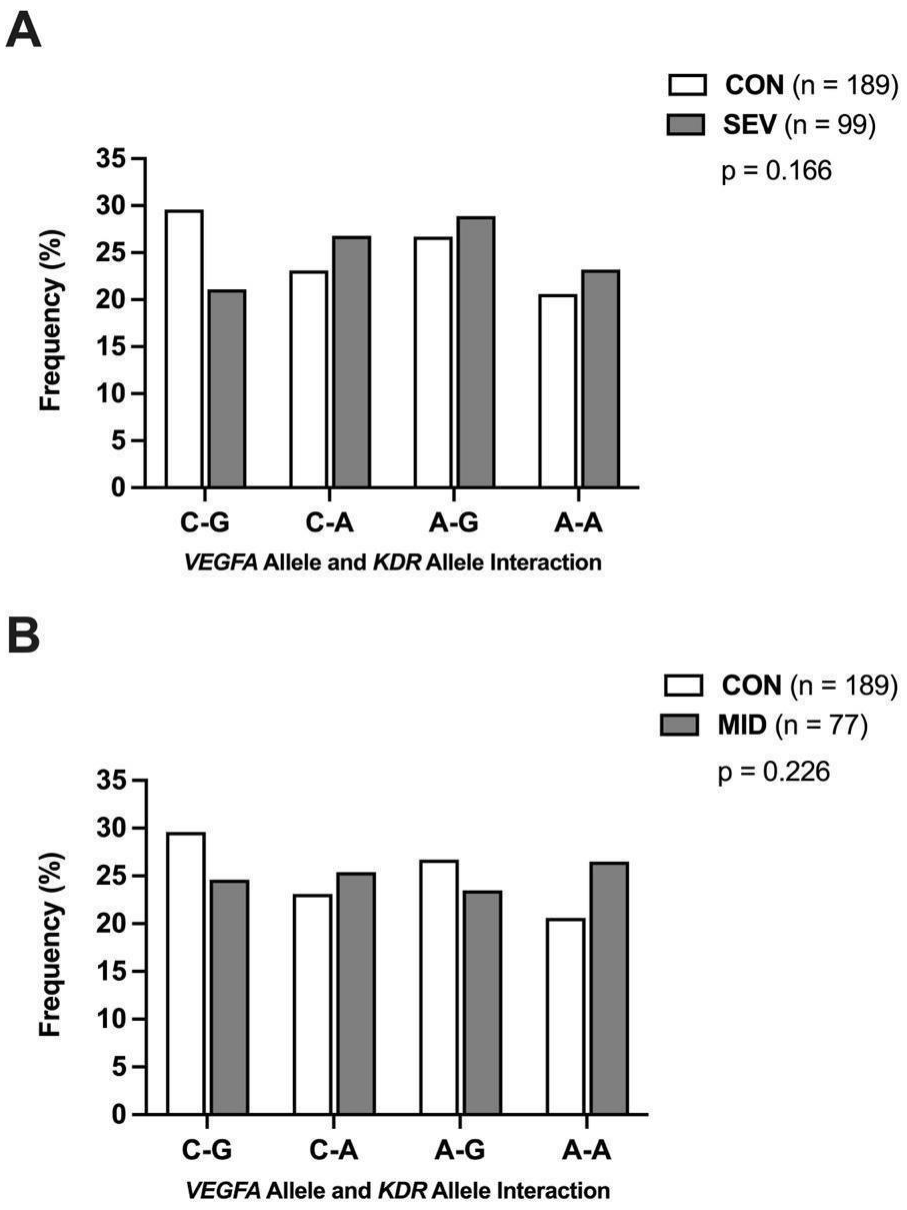

Figure 3.10: The relative frequencies of the *VEGFA* (rs699947 C>A) and *KDR* (rs2071559 G>A) allele-allele interactions in the CON (white bars) group, as well as the sub-groups of participants diagnosed with chronic Achilles tendinopathy that reported either **(A)** a grade III or grade IV injury (SEV) (grey bars) or **(B)** injury to the middle-third of the tendon (MID) (grey bars). The global recessive p-values and the number of participants (n) in each group are indicated on the graphs.

3.7 *VEGFA* and *KDR* Genotype Associations on Bilateral and Multiple Injuries

One-hundred and six (63.6%) and fifty-four (33.8%) participants of the combined TEN group reported a history of unilateral and bilateral Achilles tendinopathy, while 65.5% (n = 110) reported a history of a single injury and 34.5% (n = 58) reported a history of multiple injuries, respectively, of which 25.6% (n = 43) reported two injuries to the Achilles tendon and 8.9% (n = 15) reported three or more injuries to the Achilles tendon. Only 13.2% (n = 21) reported a history of both bilateral and multiple injuries, while 44.7% (n = 71) reported a history of a single unilateral Achilles tendinopathy. The remaining 20.8% (n = 33) and 21.4% (n = 34) reported a history of a single bilateral injury and multiple unilateral injuries, respectively.

3.7.1 *VEGFA* rs699947 (-2578 C>A) and rs2010963 (-634 G>C)

There was a significant difference in the *VEGFA* rs699947 genotype distribution between the CON group and bilateral TEN sub-group (p = 0.019, OR: 0.29, 95% CI: 0.12, 0.72), but not between the CON group and unilateral TEN sub-group (p = 0.402) (Figure 3.11A). Specifically, the CC genotype was significantly (p = 0.005) under-represented in the sub-group with a history of bilateral Achillestendinopathy (11.1%, n = 6) compared to the CON group (30.1%, n = 55). There was also a significant linear decrease in the CC genotype distribution from the CON, unilateral TEN (23.5%, n = 24) and bilateral TEN groups (linear trend p = 0.005) (Figure 3.11A).

There were no significant differences in the *VEGFA* rs699947 genotype distribution between the CON group and the TEN sub-groups with a history of a single injury (Single TEN, p = 0.142) or more than two injures (multiple TEN, p = 0.254) (Figure 3.11B). Similarly, there were no significant differences in the genotype distributions between the CON group and the single unilateral TEN (p = 0.089) sub-group, as well as between the CON group and the multiple and/or bilateral TEN (p = 0.110) sub-group (Figure 3.11C).

Finally, there were no significant differences in the *VEGFA* rs2010963 genotype distributions when the CON group was compared to the (i) unilateral and bilateral (Figure 3.11D), (ii) single injury and multiple injuries (Figure 3.11E) or (iii) single unilateral and multiple injuries and/or bilateral (Figure 3.11F) TEN sub-groups.

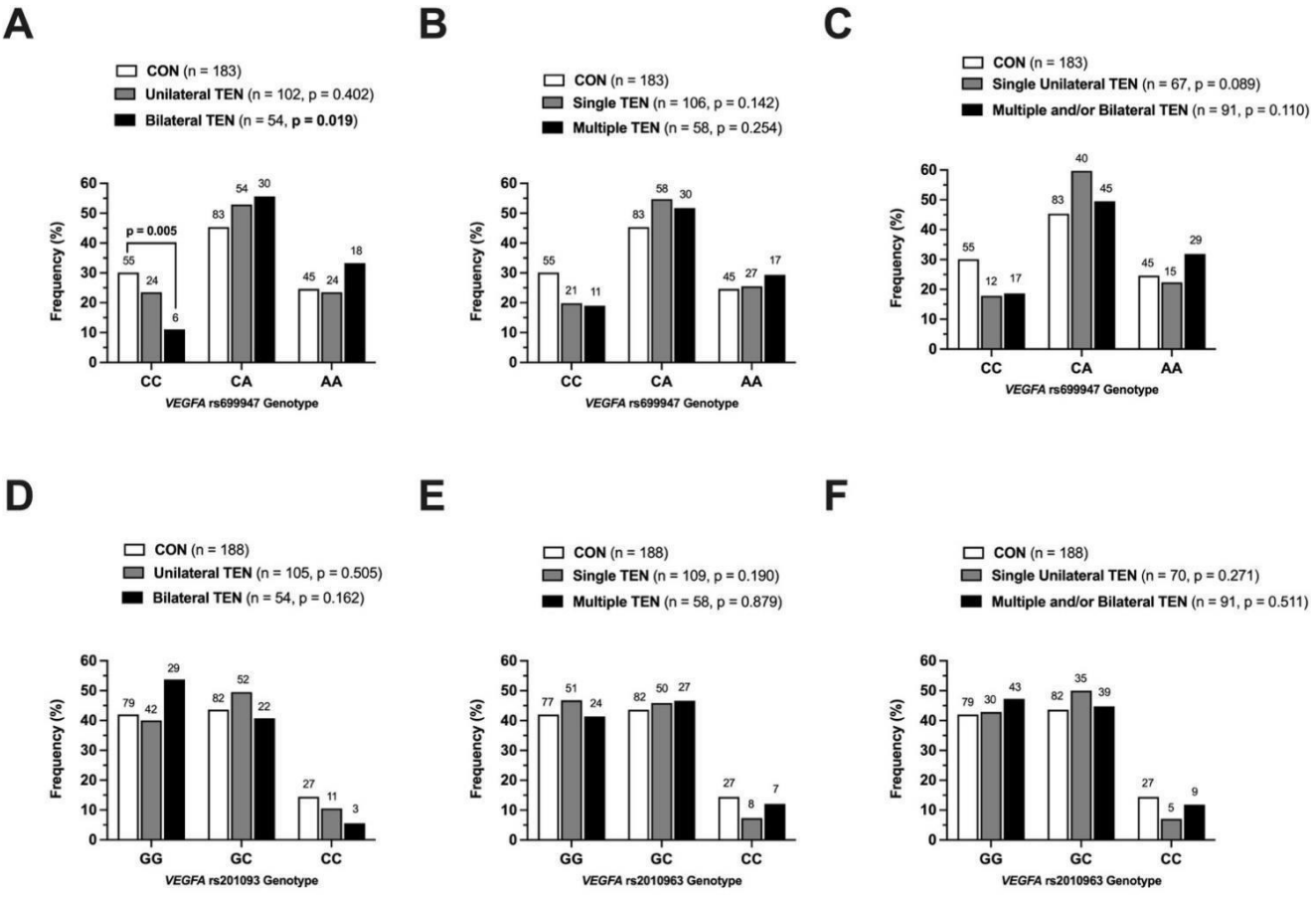

Figure 3.11: Genotype frequency distributions of the *VEGFA* **(A to C)** rs699947 (-2578 C>A) and **(D to F)** rs2010963 (-634 G>C) polymorphisms of the CON (white bars) groups compared to the **(A and D)** unilateral (grey bars) and bilateral (black bars), **(B and E)** single (grey bars) and multiple (black bars), and **(C and F)** single unilateral (grey bars) and multiple and/or bilateral (black bars) Achilles Tendinopathy (TEN) sub-groups. The number of participants (n) in each group are indicated on the graphs together with the CON vs TEN sub-group p-value. The p-value for the CON vs bilateral TEN is GG vs GC + CC. The number of participants in each genotype group and where applicable the post-hoc analysis p-values are also indicated above the bars. The p-values in bold typeset indicates significance.

3.7.2 *KDR* rs2071559 (-604 G>A) and rs1870377 (1719 T>A)

There were no significant differences in the *KDR* rs2071559 genotype distributions when the CON group was compared to the unilateral (p = 0.064) or bilateral (p = 0.252) TEN sub-groups (Figure 3.12A).

There was however a significant difference in the genotype distributions when the CON group was compared to the bilateral (p = 0.047;), but not the unilateral (p = 0.161) TEN sub-groups (Figure 3.12B). Specifically, the AA genotype was significantly under-represented (p = 0.033, OR: 2.09, 95% CI: 1.09, 4.00)in the CON group (20.1%, n = 38) compared to the multiple injury TEN sub-group (34.5%, n = 20). There was also a significant linear increase in the AA genotype distribution from the CON, single injury TEN (29.1%, n = 32) and multiple injury TEN groups (linear trend p = 0.014) (Figure 3.12B).

Similarly, there was also a significant difference in the genotype distributions when the CON group was compared to the to the multiple and/or bilateral (p = 0.016), but not the single unilateral (p = 0.400) TEN sub-groups (Figure 3.12C). Specifically, the AA genotype was significantly (p = 0.008) under-represented in the CON group (20.1%, n = 38) compared to the multiple injury and/or bilateral TEN sub-group (35.2%, n = 32). There was also a significant linear increase in the AA genotype distribution from the CON, single unilateral TEN (25.4%, n = 18) and multiple injury and/or bilateral TEN sub-groups (linear trend p = 0.007) (Figure 3.12C).

Finally, there were no significant differences in the *KDR* rs1870377 genotype distributions when the CON group was compared to the (i) unilateral and bilateral (Figure 3.12D), (ii) single and multiple injury (Figure 3.12E) or (iii) single unilateral and multiple injury and/or bilateral (Figure 3.12F) TEN sub-groups.

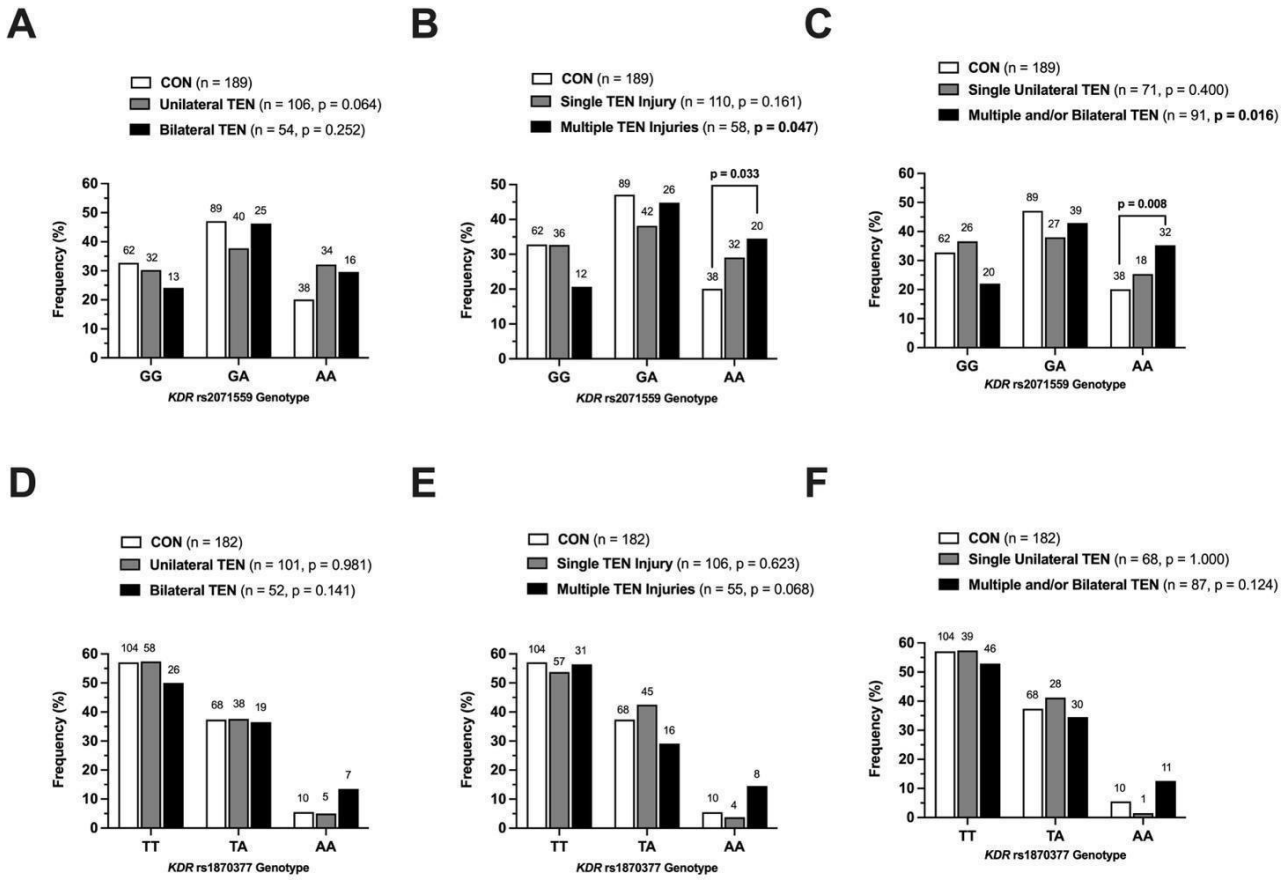

Figure 3.12: Genotype frequency distributions of the *KDR* **(A to C)** rs2071559 (-604 G>A) and **(D to F)** rs1870377 (1719 T>A) polymorphisms of the CON (white bars) groups compared to the **(A and D)** unilateral (grey bars) and bilateral (black bars), **(B and E)** single (grey bars) and multiple (black bars), and **(C and F)** single unilateral (grey bars) and multiple and/or bilateral (black bars) Achilles Tendinopathy (TEN) sub-groups. The number of participants (n) in each group are indicated on the graphs together with the CON vs TEN sub-group p-value. The p-value for the CON vs Single TEN and CON vs Single Unilateral TEN is TT vs TA + AA. The number of participants in each genotype group and where applicable the post-hoc analysis p-values are also indicated above the bars. The p-values in bold typeset indicates significance.

3.8 *VEGFA* and *KDR* Haplotype and Allele Interaction Associations on Bilateral and Multiple Injuries

There were no significant differences in the relative frequencies of the four inferred haplotypes constructed from the *VEGFA* rs699947 C>A and rs2010963 G>C polymorphisms between the CON group and unilateral TEN sub-group (dominant model p = 0.303) (Figure 3.13A). There was however a significant difference in the relative frequencies of the inferred haplotypes when the CON group was compared to the bilateral TEN sub-group (dominant model p = 0.042, OR: 2.80, 95% CI: 1.04, 7.50), specifically the A-G inferred haplotype was significantly over-represented in the bilateral TENsub-group (45.4% CON vs 60.0% bilateral TEN, p = 0.005) (Figure 3.13A). There were however no significant differences in the relative frequencies of the inferred *VEGFA* haplotypesbetween the CON group and unilateral TEN sub-group (dominant model p = 0.175) or CON group and unilateral TEN sub-group (dominant model p = 0.354) (Figure 3.13B). Similarly, there were also no significant differences in the relative frequencies of the inferred *VEGFA* haplotypes between the CON group and single unilateral injury TEN sub-group (dominant model p = 0.116) or CON group compared to the multiple and/or bilateral injury TEN sub- group (dominant model p = 0.256) (Figure 3.13C).

There were no significant differences in the relative frequencies of the four inferred haplotypes constructed for the *KDR* rs2071559 G>A and rs1870377 T>A polymorphisms between the CON group and unilateral TEN sub-group (additive model p = 0.359) or the CON group and bilateral TEN sub-group (additive model p = 0.185) (Figure 3.13D). Similarly, there were no significant differences in the relative frequencies of the *KDR* inferred haplotypes between the CON group and single injury TEN sub-group (additive model p = 0.543) or between the CON and multiple injury TEN groups (additive model p = 0.087) (Figure 3.13E). Although there were no significant differences in the relative frequencies of the *KDR* inferred haplotypes between the CON group and single unilateral injury TEN sub-group (additive model p = 0.955),there were significant differences when the CON group was compared to the multiple and/or bilateral injury TEN sub-group (additive model p = 0.047, OR: 0.43, 95% CI: 0.23, 0.43) (Figure 3.13F). Specifically, the G-T inferred haplotype was significantly (p = 0.028, OR: 0.51; 95% CI: 0.31, 0.85) under-represented in the TEN sub-group (34.1%) compared to the CON group (42.8%), while the A-A inferred haplotype was significantly (p = 0.0 12) over-represented in the TEN sub-group (20.7%) compared to the CONgroup (11.8%) (Figure 3.13F).

There were no significant differences in the relative distributions of the four *VEGFA* rs699947 allele and *KDR* rs2071559 allele interactions between the CON group and the unilateral TEN sub-group (Figures 3.14A), between the CON group and the single unilateral TEN sub-group (Figures 3.14B), as well as the CON group and the multiple and/or bilateral TEN sub-group (Figures 3.14C). There were significant differences in the relative distributions of the allele-allele interactions when the CON group was compared to the bilateral TEN subgroup (additive model p = 0.029, OR: 0.41, 95% CI:0.22, 0.76) and recessive model p = 0.006) (Figures 3.14A). Specifically, the C-G allele- allele interaction was significantly under-represented (additive model p = 0.018 and recessive model p = 0.008) in the bilateral TEN sub-group (18.7%) compared to the CON group (29.6%),while the A-A allele-allele interaction was significantly over-represented (additive model p = 0.005 and recessive model p = 0.006) in the bilateral TEN sub-group (32.6%) compared to the CON group (20.6%) (Figure 3.14A). There were also significant differences in the relative distributions of the allele-allele interactions between the CON and multiple injury TEN sub- groups (additive model p = 0.046 and recessive model p = 0.100) (Figures 3.14B). The C-G allele-allele interaction was significantly under-represented (additive model p = 0.016) in the multiple injury TEN sub-group (22.9%) compared to the CON group (29.6%), while the A-A allele-allele interaction was significantly over-represented in the (additive model p = 0.013) multiple injury TEN sub-group (24.0%) compared to the CON group (20.6%) (Figure 3.14B). Similarly, there were also significant differences in the relative distributions of the allele-allele interactions between the CON and multiple and/or bilateral injury TEN groups (additive modelp = 0.037, OR: 0.48, 95% CI: 0.29, 0.80) and recessive model p = 0.089) (Figures 3.14C). The C-G allele-allele interaction was significantly under-represented (additive model p = 0.008) in the multiple injury TEN sub-group (17.1%) compared to the CON group (27.7%), while the A-A allele-allele interaction was significantly over-represented (additive model p = 0.014; OR: 0.36, 95% CI: 0.16, 0.83) in the multiple injury TEN sub-group (30.3%) compared to the CON group (21.8%) (Figure 3.14C).

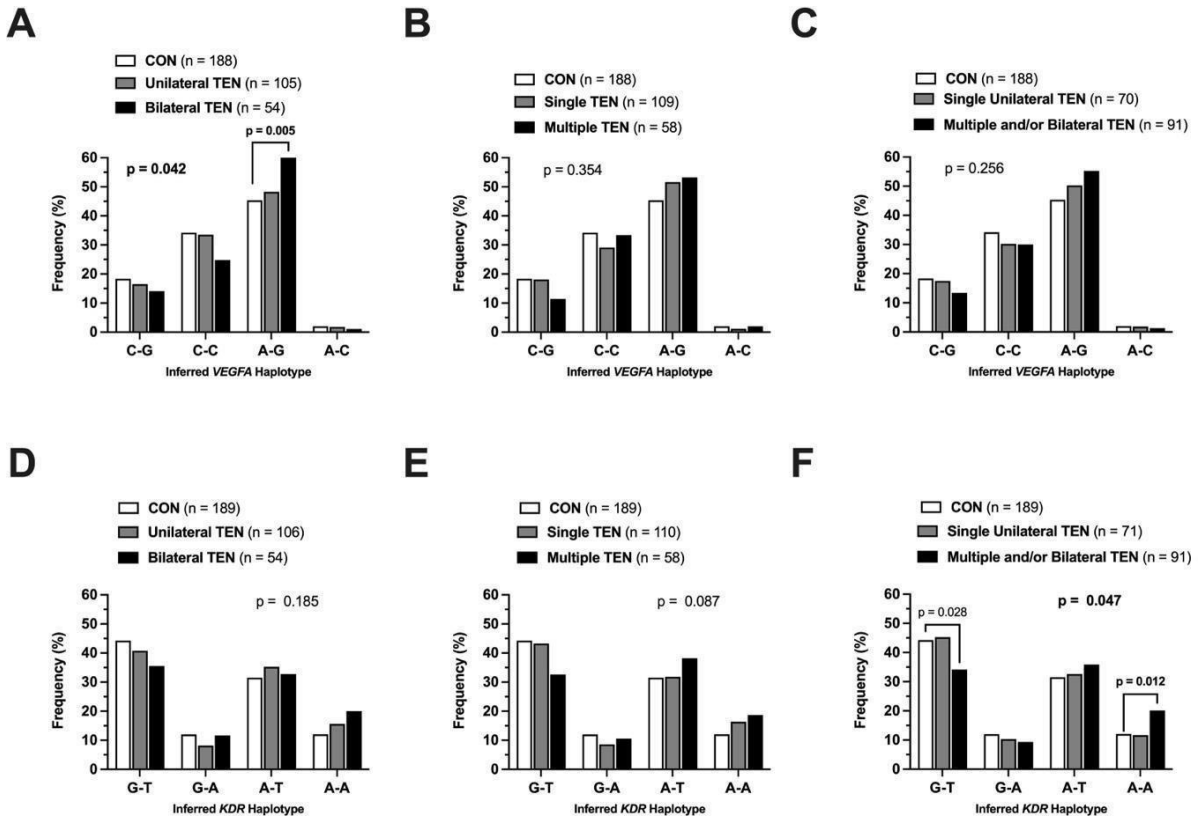

Figure 3.13: The relative frequencies of the inferred haplotypes constructed from **(A to C)** *VEGFA* (rs699947 C>A and rs2010963 G>C), as well as the **(D to F)** *KDR* (rs2071559 G>A and rs1870377 T>A) polymorphisms for the CON (white bars) groups compared to the **(A and D)** unilateral (grey bars) and bilateral (black bars), (**B** and **E**) single (grey bars) and multiple (black bars) injuries, and **(C and F)** single unilateral (grey bars) and multiple injury and/or bilateral (black bars) Achilles Tendinopathy (TEN) subgroups. The global dominant (CON vs Bilateral TEN, CON vs Multiple TEN, and CON vs Multiple and/or Bilateral TEN) and additive (CON vs Bilateral TEN, CON vs Multiple TEN, and CON vs Multiple and/or Bilateral TEN) p-values for the inferred *VEGFA* and *KDR* haplotypes, respectively, and the number of participants (n) in each group is indicated on the graphs. Where applicable the post-hoc analysis p-values are also indicated above the inferred haplotype bars. The p-values in bold typeset indicates significance.

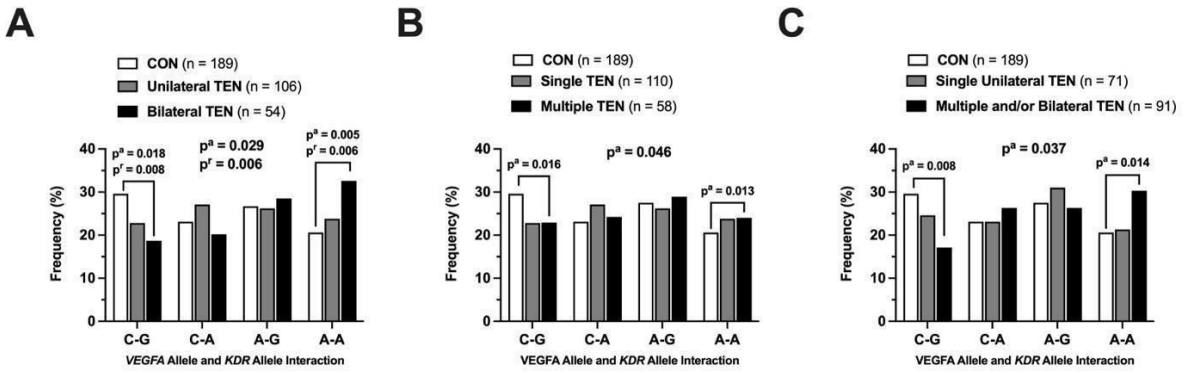

Figure 3.14: The relative distributions of the *VEGFA* (rs699947 C>A) and *KDR* (rs2071559 G>A) allele-allele interactions for the CON (white bars) groups compared to the **(A)** unilateral (grey bars) and bilateral (black bars), (**B**) single (grey bars) and multiple (black bars), and **(C)** single unilateral (grey bars) and multiple and/or bilateral (black bars) Achilles Tendinopathy (TEN) subgroups. The global p-values (additive model of inheritance (p^a) and/or recessive (p^r)) for the **(A)** CON vs Bilateral TEN, **(B)** CON vs Multiple TEN and **(C)** CON vs Multiple and/or Bilateral TEN p-values for the allele-allele interactions and the number of participants (n) in each group is indicated on the graphs. Where applicable the post-hoc analysis p-values (p^a and/or p^r) are also indicated above the allele-alleleinteraction bars. The p-values in bold typeset indicates significance.

Chapter 4

Association of the *VEGFA* and *KDR* Genes with Ultrasound and Multidimensional Pain Measurements in Athletes with Chronic mid-portion Achilles Tendinopathy

4. 1 Participant characteristics

Seventy-seven of the previously described participants with either unilateral or bilateral (n = 21) chronic Achilles Tendinopathy (TEN) who had completed the multidimensional pain scales (VISA-A, sf-BPI and sf-MPQ questionnaires) and whose Achilles tendons were examined using ultrasound and colour Doppler were included in this chapter (23,24). The median (Q1, Q3) age at the time of first injury, height, weight at recruitment and BMI at recruitment of the TEN participants were 43.0 (32.3; 50.0) years, 174.0 (168.0; 178.0) centimetres, 74.0 (65.0. 84.0) kg and 24.3 (21.8; 26.8) $kg.m^{-2}$, respectively. Forty-five (58.4%) of the TEN participants were male. Sixty (77.9%) of the participants reported that running was their main sport.

4.2 *VEGFA* and *KDR* Genotype Associations on Participant Characteristics

There were no significant *VEGFA* rs699947 and *KDR* rs1870377 genotype associations on the physical characteristics of the TEN participants (Supplementary Tables S4.1 and S4.4). Although matched for height, weight and BMI, the *VEGFA* rs2010963 GC genotype (37.1 ± 11.7 years, n = 32) group was on average significantly younger at the time of injury compared to the GG (45.3 ± 10.2 years, n = 36, P = 0.004), but not the CC genotype (44.6 ± 14.5 years, n = 8, P = 0.095) groups (Supplementary Table S4.2). In addition, there were significantly more males in the GG genotype (75.7%, P = 0.006) group compared to the combined GC and CC genotype groups (43.9%) (Supplementary Table S4.2). The *KDR* rs2071559 GA genotype (79.3 ± 14.5 kg, n = 31) group was significantly heavier than the AA (70.5 ± 11.8 kg, n = 24, P = 0.018) but not the GG (72.1 ± 13.2 kg, n = 20, P = 0.064) genotype groups (Supplementary table S4.3). The *VEGFA* rs699947 (P = 0.819), *VEGFA* rs2010963 (P = 0.793), *KDR* rs2071559

(P = 0.109) and *KDR* rs1870377 (P = 0.124) polymorphisms were all in Hardy-Weinberg Equilibrium (HWE).

4.3 *VEGFA* and *KDR* Genotype Associations on Achilles Tendon Diameters

VEGFA and *KDR* genotype associations on the Achilles tendon diameters with bilateral tendinopathies were analysed by comparing the diameters of the tendon in the dominant leg with the tendon with the largest diameter (Supplementary Tables S4.5). Since the diameters of the genotype groups were similar, the Achilles tendon with the largest diameter for participants with bilateral tendinopathy was included as the injured tendon for further analysis.

Although the injured tendon generally had a larger diameter, there were no significant differences in the diameters of the injured and uninjured tendons between the *VEGFA* rs699947, *VEGFA* rs2010963 and *KDR* 1870377 genotype groups (Table 4.1). The median injured (P < 0.001) and uninjured (P = 0.004) Achilles tendon diameters of the *KDR* rs2071559 GA genotype group were significantly larger than the GG and AA genotype groups (Table 4.1). However, there were no significant differences in the injured (P = 0.093) and uninjured (P = 0.105) diameters between the genotype groups when corrected for the differences in participantweight (Table 4.1 and Supplementary table S4.3). Weight at recruitment of the TEN participantswas positively correlated with the diameter of the injured tendon (r = 0.294, P = 0.082, n = 36) but not the uninjured tendon (r = 0.317, P = 0.022, n = 52).

4.4 *VEGFA* and *KDR* Genotype Associations on Achilles Tendon Pathology

The *VEGFA* and *KDR* genotype associations on Achilles tendon pathology pertaining to bilateral tendinopathy were analysed by comparing the abnormal ultrasound findings of the tendon in the dominant leg with those of the tendon with the largest abnormal ultrasound findings (Supplementary Table S4.6). Since the abnormal ultrasound findings of the genotype groups were similar, the Achilles tendon with the largest diameter for participants with bilateral tendinopathy was included as the injured tendon for further analysis.

Table 4.1: *VEGFA* rs699947 (-2578 C>A), *VEGFA* rs2010963, (-634 G>C), *KDR* rs2071559 (-604 G>A) and *KDR* rs1870377 (1719 T>A) genotype associations on the diameter of the injured and uninjured Achilles tendons within the chronic Achilles tendinopathy (TEN) group.

			Injured Tendon			Uninjured Tendon	
SNP	**Genotype**	**n**	**Diameter** (mm)	**P-value**	**n**	**Diameter** (mm)	**P-value**
***VEGFA* rs699947**	**CC**	15	7.6 ± 2.7		14	6.0 (4.9; 7.3)	
	CA	21	7.4 ± 2.1	0.939	11	5.5 (4.9; 7.3)	0.494
	AA	17	7.3 ± 2.2		12	6.8 (5.3; 7.5)	
***VEGFA* rs2010963**	**GG**	25	6.9 (5.5; 8.6)		15	6.4 (5.3; 7.5)	
	GC	22	6.8 (5.4; 8.4)	0.570	16	5.3 (4.8; 7.1)	0.117
	CC	6	8.1 (6.5; 10.1)		6	7.2 (6.6; 7.7)	
***KDR* rs2071559**	**GG**	11	6.1 (5.4; 7.1)		7	5.5 (5.2; 7.1)	
	GA	23	8.1 (7.2; 9.7)	0.093 [1]	14	7.3 (7.1; 7.8)	0.105 [1]
	AA	19	5.4 (4.9; 7.4)	**(<0.001)**	16	5.2 (4.8; 6.4)	**(0.004)**
***KDR* rs1870377**	**TT**	30	7.2 (6.2; 8.7)		21	6.8 (5.1; 7.3)	
	TA	17	6.1 (5.4; 9.8)	0.498	13	5.9 (4.9; 7.5) [2]	0.774
	AA	5	7.4 (4.7; 8.1)		3		

Values are expressed as average ± standard deviation or median (Q1; Q3) for parametric and non-parametric data

The larger tendon diameter was used for participants with bilateral Achilles tendinopathy

P-values in parenthesis and bold typeset indicates uncorrected significance

SNP, single nucleotide polymorphism; *VEGFA*, Vascular endothelial growth factor A*; KDR*, Kinase Domain Receptor

[1]Corrected for weight (weight/diameter)

[2]TA + AA

There were no significant differences in the relative number of abnormal ultrasound findings of the injured and uninjured tendons between the *VEGFA* rs699947, *VEGFA* rs2010963 and *KDR* rs1870377 genotype groups in the TEN participants (Table 4.2). There were however significant differences in the absence (yes) and presence (no) of abnormal ultrasound findings of the injured, but not the uninjured tendon between the *KDR* rs2071559 genotype groups (Table 4.2). Specifically, the GA genotype group, which was significantly heavier than the other genotype groups (Supplementary Table S4.3), was significantly over-represented in the participants with abnormal findings of the injured tendon ($P = 0.041$). Although not statistically significant ($P = 0.064$), participants with abnormal findings of the injured tendon were heavier (77.6 ± 16.5 kg, $n = 32$) than those with no abnormal findings (71.0 ± 10.7 kg, $n = 31$).

Table 4.2: *VEGFA* rs699947 (-2578 C>A), *VEGFA* rs2010963, (-634 G>C), *KDR* rs2071559 (-604 G>A) and *KDR* rs1870377 (1719 T>A) genotype associations on the presence (Yes) and absence (No) of abnormal ultrasound findings in injured and uninjured tendons of TEN participants.

		Injured Tendon			Uninjured Tendon		
SNP	**Genotype**	**Yes**	**No**	**P-value**	**Yes**	**No**	**P-value**
***VEGFA* rs699947**	**CC**	23.5 (8)	25.8 (8)		27.8 (5)	34.5 (10)	
	CA	50.0 (17)	38.7 (12)	0.630	38.9 (7)	34.5 (10)	0.889
	AA	26.5 (9)	35.5 (11)		33.3 (6)	31.0 (9)	
***VEGFA* rs2010963**	**GG**	44.1 (15)	51.2 (16)		50.5 (9)	41.4 (12)	
	GC	44.1 (14)	41.9 (13)	0.803 [1]	33.3 (6)	48.3 (14)	0.763 [1]
	CC	11.8 (4)	6.5 (2)		16.7 (3)	10.3 (3)	
***KDR* rs2071559**	**GG**	26.5 (9)	29.0 (9)		16.7 (3)	31.0 (9)	
	GA	52.9 (18)	25.8 (8)	**0.048**	55.6 (10)	24.1 (7)	0.059 [2]
	AA	20.6 (7)	45.2 (14)		27.8 (5)	44.8 (13)	
***KDR* rs1870377**	**TT**	66.7 (22)	54.8 (17)		61.1 (11)	62.1 (18)	
	TA	24.2 (8)	35.5 (11)	0.443 [3]	38.9 (7)	27.6 (8)	1.000 [3]
	AA	9.1 (3)	9.7 (3)		0.0 (0)	10.3 (3)	

Values are expressed as a percentage with the number of participants (n) in parentheses

The tendon with the larger abnormal ultrasound findings was used for participants with bilateral Achilles tendinopathy

SNP, single nucleotide polymorphism; *VEGFA*, Vascular endothelial growth factor A; *KDR*, Kinase Domain Receptor

P-values in bold typeset indicates significance

[1]GG vs GC and CC

[2]GA vs GG and AA

[3]TT vs TA and AA

4.5 *VEGFA* and *KDR* Genotype Associations on Self-Reported Achilles Tendon Pain

There were no significant differences in the VISA-A, sf-MPQ and sf-BPI, as well as the subscale scores between the *VEGFA* rs699947 genotype groups (Table 4.3). Similarly, there were no significant differences in the VISA-A and sf-MPQ, as well the subscale scores between the *VEGFA* rs2010963 genotype groups (Table 4.4).

However, except for the sf-BPI interference subscale scores, there were *VEGFA* rs2010963 genotype group differences between the sf-BPI total and sf-BPI intensity subscale scores (Table 4.4). The GC genotype (Supplementary Table S4.2), which contained more younger females, had higher scores. The median (Q1; Q3) of the female's sf-BPI total score (24.0 (12.8; 38.5), n = 30) was significantly higher (P = 0.002) than the male's score (11.0 (6.0; 21.0), n = 45) and the intensity subscale was also significantly higher (P = 0.015) in females (11.0 (7.0; 15.0), n = 30) compared to males (7.0 (4.0; 12.0), n = 45). There was however no significant correlation between age at recruitment, sf-BPI total (r = -0.124, P = 0.289, n = 75) and sf-BPI intensity subscale (r = -0.134, P = 0.252, n = 75) scores.

Finally, there were no significant differences in the VISA-A, sf-MPQ and sf-BPI, as well the subscale, scores between the *KDR* rs2071559 and rs1870377 genotype groups (Tables 4.5 and 4.6).

Table 4.3 *VEGFA* rs699947 (-2578 C>A) genotype associations on the participants with chronic Achilles tendinopathy (TEN) (i) Victorian Institute Sports Assessment – Achilles (VISA-A), (ii) self-reported tendon pain from the short form McGill Pain Questionnaire (sf-MPQ), and (iii) short form Brief Pain Inventory (sf-BPI).

	CC (n = 18)	**CA** (n = 37)	**AA** (n = 22)	**P-value**
VISA-A Total	75.3 ± 13.5	67.6 ± 17.6	70.5 ± 15.8	0.277
VISA-A Sensory Subscale	37.0 (33.0; 47.5)	39.0 (31.0; 45.8)	38.0 (35.0; 47.3)	0.933
VISA-A Interference Subscale	34.0 (28.0; 45.5)	29.5 (24.3; 36.0)	36.5 (17.0; 40.0)	0.207
sf-MPQ Total	6.1 (4.0; 9.0)	5.6 (3.0; 12.0)	7.4 (3.8;12.0)	0.909
PPI	1.0 (0.0; 2.0)	1.0 (0.0; 2.0)	1.0 (0.0; 2.0)	0.933
VAS	0.1 (0.0; 1.9)	0.0 (0.0; 2.7)	1.5 (0.0; 3.6)	0.367
sf-MPQ Sensory Subscale	3.0 (2.5; 5.5)	3.0 (2.0; 6.8)	3.0 (2.0; 6.0)	0.988
sf-BPI Total	14.0 (11.0; 28.5)	18.0 (6.5; 26.5)	10.5 (6.0; 25.3)	0.362
sf-BPI Intensity Subscale	7.0 (5.5; 14.0)	10.0 (6.0; 14.0)	7.5 (2.8; 12.0)	0.260
sf-BPI Interference Subscale	10.0 (1.5; 14.0)	6.5 (1.3; 14.8)	4.0 (1.0; 11.3)	0.589

Values are reported as average ± standard deviation or median (Q1; Q3) for parametric and non-parametric data respectively

The number of participants (n) in each genotype group is indicated

VEGFA, Vascular endothelial growth factor A; PPI, present pain index; VAS, visual analogue scale

Table 4.4 *VEGFA* rs2010963 (-634 G>C) genotype associations on the participants with chronic Achilles tendinopathy (TEN) (i) Victorian Institute Sports Assessment – Achilles (VISA-A), (ii) self-reported tendon pain from the short form McGill Pain Questionnaire (sf-MPQ), and (iii) short form Brief Pain Inventory (sf-BPI).

	GG (n = 37)	**GC** (n = 32)	**CC** (n = 8)	**P-value**
VISA-A Total	71.2 ± 16.0	68.2 ± 17.2	71.0 ± 15.5	0.675
VISA-A Sensory Subscale	39.0 (35.0; 48.0)	38.0 (30.0; 45.0)	36.5 (29.5; 49.5)	0.196
VISA-A Interference Subscale	31.3 ± 10.9	31.3 ± 14.1	32.4 ± 7.8	0.971
sf-MPQ Total	5.3 (3.0; 9.9)	7.4 (4.0; 11.2)	8.1 (4.3;16.5)	0.320
PPI	1.0 (0.0; 2.0)	1.0 (0.0; 2.0)	1.5 (0.0; 2.0)	0.391
VAS	0.6 (0.0; 2.5)	0.9 (0.0; 3.4)	0.0 (0.0; 1.5)	0.509
sf-MPQ Sensory Subscale	3.0 (2.0; 5.0)	3.0 (2.0; 6.8)	7.0 (1.8; 11.0)	0.200
sf-BPI Total	10.0 (6.0; 22.5)	20.0 (11.0; 29.0)	15.5 (11.8; 47.0)	**0.023** [1]
sf-BPI Intensity Subscale	6.5 (3.3; 11.8)	10.0 (7.0; 14.0)	9.5 (6.0; 22.3)	**0.040** [2]
sf-BPI Interference Subscale	3.0 (1.0; 11.8)	11.0 (2.9; 14.0)	10.5 (2.8; 24.8)	0.166

Values are reported as average ± standard deviation or median (Q1; Q3) for parametric and non-parametric data respectively

The number of participants (n) in each genotype group is indicated

VEGFA, Vascular endothelial growth factor A; PPI, present pain index; VAS, visual analogue scale

P-values in bold typeset indicates significance

[1]Post hoc analysis: GG vs GC **P = 0.046**; GG vs CC P = 0.164; GC vs CC P = 1.000

[2]Post hoc analysis: GG vs GC P = 0.062; GG vs CC P = 0.282; GC vs CC P = 1.000

Table 4.5: *KDR* rs2071559 (-604 G>A) genotype associations on the participants with chronic Achilles tendinopathy (TEN) (i) Victorian Institute Sports Assessment – Achilles (VISA-A), (ii) self-reported tendon pain from the short form McGill Pain Questionnaire (sf-MPQ), and (iii) short form Brief Pain Inventory (sf-BPI).

	GG (n = 22)	**GA** (n = 31)	**AA** (n = 24)	**P-value**
VISA-A Total	68.9 ± 15.9	69.7 ± 15.9	72.0 ± 15.2	0.796
VISA-A Sensory Subscale	39.0 (33.0; 48.0)	37.5 (34.0; 46.3)	39.0 (34.3; 46.8)	0.834
VISA-A Interference Subscale	30.1 ± 12.6	31.1 ± 11.8	32.2 ± 12.0	0.928
sf-MPQ Total	4.1 (2.8; 10.3)	7.2 (4.0; 12.0)	7.0 (3.3;10.5)	0.402
PPI	1.0 (0.0; 2.0)	1.0 (0.0; 2.0)	1.0 (1.0; 2.0)	0.733
VAS	0.3 (0.0; 2.3)	0.3 (0.0; 2.3)	0.7 (0.0; 3.2)	0.865
sf-MPQ Sensory Subscale	3.0 (1.8; 6.0)	4.0 (2.0; 7.0)	3.0 (1.3; 5.8)	0.407
sf-BPI Total	20.0 (7.0; 33.5)	13.5 (7.8; 25.5)	14.5 (6.3; 25.0)	0.837
sf-BPI Intensity Subscale	9.9 ± 6.3	9.9 ± 6.5	8.9 ± 5.2	0.806
sf-BPI Interference Subscale	11.0 (1.5; 15.5)	2.0 (1.0; 12.8)	6.0 (2.3; 13.4)	0.574

Values are reported as average ± standard deviation or median (Q1; Q3) for parametric and non-parametric data respectively

The number of participants (n) in each genotype group is indicated

KDR, Kinase Domain Receptor; PPI, present pain index; VAS, visual analogue scale

Table 4.6: *KDR* rs1870377 (1719 T>A) genotype associations on the participants with chronic Achilles tendinopathy (TEN) (i) Victorian Institute Sports Assessment – Achilles (VISA-A), (ii) self-reported tendon pain from the short form McGill Pain Questionnaire (sf-MPQ), and (iii) short form Brief Pain Inventory (sf-BPI).

	TT (n = 46)	**TA** (n = 23)	**AA** (n = 7)	**P-value**
VISA-A Total	69.4 ± 14.0	72.4 ± 19.7	71.3 ± 19.1	0.778
VISA-A Sensory Subscale	38.0 (33.5; 47.0)	39.0 (33.8; 46.0)	45.0 (28.0; 49.0)	0.774
VISA-A Interference Subscale	30.0 (25.0; 38.0)	37.0 (29.3; 43.3)	28.0 (24.0; 47.0)	0.185
sf-MPQ Total	5.6 (4.0; 11.5)	6.4 (3.9; 11.2)	7.5 (2.7;14.0)	0.975
PPI	1.0 (0.0; 2.0)	1.0 (0.0; 2.0)	1.0 (0.0; 2.0)	0.923
VAS	0.0 (0.0; 2.4)	1.5 (0.0; 2.1)	0.7 (0.0; 5.0)	0.427
sf-MPQ Sensory Subscale	3.0 (2.0; 7.0)	3.0 (2.0; 6.0)	2.0 (1.0; 8.0)	0.770
sf-BPI Total	17.0 (7.5; 27.5)	14.0 (6.0; 25.3)	15.0 (7.0; 41.0)	0.733
sf-BPI Intensity Subscale	9.0 (5.5; 12.5)	7.5 (4.8; 12.3)	9.0 (6.0; 19.0)	0.605
sf-BPI Interference Subscale	6.0 (1.0; 14.5)	5.0 (1.0; 11.5)	6.0 (4.0; 21.0)	0.764

Values are reported as average ± standard deviation or median (Q1; Q3) for parametric and non-parametric data respectively

The number of participants (n) in each genotype group is indicated

KDR, Kinase Domain Receptor; PPI, present pain index; VAS, visual analogue scale

Chapter 5

Discussion

The main novel findings of this dissertation was that although the *VEGFA* and *KDR* polymorphisms were not associated with chronic Achilles tendinopathy in a combined larger cohort, (i) the *VEGFA* rs699947 CC genotype was significantly associated with decreased risk of bilateral chronic Achilles tendinopathy, (ii) the A-G inferred *VEGFA* haplotype constructed from rs699947 and rs2010963 was associated with increased risk of bilateral tendinopathy, (iii) the *KDR* rs2071559 AA genotype was significantly associated with increased risk of a history of multiple (two or more) chronic Achilles tendinopathies, (iv) the G-T and A-A *KDR* inferred haplotypes constructed from rs2071559 and rs1870377 were associated with decreased and increased risk of multiple and/or bilateral chronic Achilles tendinopathies, respectively, and (v) the C-G and A-A *VEGFA* rs699947 and *KDR* rs2071559 allele-allele interactions were significantly associated with decreased and increased risk of bilateral or multiple injuries respectively. Finally, the *VEGFA* and *KDR* polymorphisms were not associated with ultrasound and multidimensional pain measurements in participants with chronic Achilles tendinopathy.

5.1 *VEGFA* and *KDR* polymorphisms are not associated with chronic Achilles tendinopathy

A candidate gene case-control genetic association study approach was employed in Chapter 3 of this dissertation to further investigate the association of functional polymorphisms within the *VEGFA* and *KDR* genes with respect to chronic Achilles tendinopathy in a combined larger cohort consisting of two previously described South African physically active groups (24,50). The first set of findings of the study was that the *VEGFA* rs699947 (-2578 C>A), *VEGFA* rs2010963 (-634 G>C), *KDR* rs2071559 (-604 G>A) and *KDR* rs1870377 (1719 T>A) genotypes and alleles were not independently associated with chronic Achilles tendinopathy in the combined South African cohort. Similarly, the genotypes and alleles were not associated with chronic Achilles tendinopathy when the male and female participants were analysed separately. Additionally, there were no associations for the inferred *VEGFA* or *KDR* haplotypes constructed from the variants with chronic Achilles tendinopathy in the combined as well as in the male and female participants.

Contrary to this, the *VEGFA* rs699947 CC genotype was previously reported to be significantly associated with reduced risk of chronic Achilles tendinopathy in one of the South African cohorts included in this analysis, but not in a British cohort (50) (Table 5.1). Although variant rs2010963 was not independently associated with increased risk, the A-G-G inferred haplotype constructed from the *VEGFA* rs699947, rs1570360 (-1154 G>A), and rs2010963 variants were also previously associated with increased risk of chronic Achilles tendinopathy in the South African, but not the British cohorts (Table 5.1). Due to technical reasons, the second South African cohort included in this dissertation could not be genotyped for rs1570360 (24). Therefore, inferred haplotypes consisting of all three *VEGFA* variants could not be constructed in the larger combined South African cohort. Similar to the results presented in this dissertation, Rahim (50) reported that the two *KDR* variants, as well as the inferred *KDR* haplotypes were not associated with chronic Achilles tendinopathy in the South African and British cohorts (Table 5.1).

VEGFA rs699947, together with two additional variants, rs833061 (-460 T>C) and rs3025039 (935 C>T), not investigated in this dissertation, were also reportedly not independently associated with patellar, shoulder and Achilles tendinopathy in Brazilian volleyball athletes (10) (Table 5.1). The *KDR* rs2305948 (1192 G>A) GG genotype was associated with increased risk of tendinopathy, while the *KDR* rs2071559 and rs1870377 variants were not associated with tendinopathy in Brazilian volleyball athletes (Table 5.1). Both the *KDR* G-G-A and G-A-T inferred haplotypes constructed from rs2071559, rs2305948 and rs1870377 were associated with decreased tendinopathy risk (10) (Table 5.1). The nonsynonymous *KDR* rs2305948 variant, which has a valine to isoleucine change at amino acid position 297 within exon 7, was not analysed in this dissertation and should therefore be included in future studies.

5.2 *VEGFA* and *KDR* polymorphisms are associated with bilateral Achilles tendinopathy

It is not unusual for initial genetic associations with multifactorial conditions, including musculoskeletal soft tissue injuries, not to be repeated in follow-up studies (58). Although there are several reasons for this, heterogeneity of the clinical phenotype is one possibility. This and other published tendinopathy cohorts consisted of unilateral and bilateral injuries, single and multiple injuries, grades I to IV injuries and different injured regions within the tendon. Therefore, the association of the *VEGFA* and *KDR* variants with regards to the specific region

of injury, severity of injury, and number of injuries were further investigated in Chapter 3 of this study.

The second set of findings of this study was that the *VEGFA* and *KDR* genotypes and alleles were not independently associated with chronic Achilles tendinopathy in participants who reported either a grade III or grade IV injury nor an injury specifically to the middle-third of the tendon. Similarly, the inferred *VEGFA* or *KDR* haplotypes were also not associated with grade III or grade IV chronic Achilles tendinopathy nor an injury to the middle-third of the tendon. However, the *VEGFA* rs699947 CC genotype was significantly associated with decreased risk of bilateral chronic Achilles tendinopathy in the larger combined South Africancohort (Table 5.1). Interestingly, although not associated with unilateral tendinopathy, there was also a significant linear decrease in the CC genotype distribution when the control, unilateral Achilles tendinopathy and bilateral Achilles tendinopathy groups were compared. The *VEGFA* rs699947 variant, specifically the CC genotype was not associated with a history of either single or multiple injuries. Although the *VEGFA* rs2010963 variant was not independently associated with unilateral, bilateral, single or multiple Achilles tendinopathies, the A-G inferred *VEGFA* haplotype constructed from rs699947 and rs2010963 was associated with increased risk of bilateral, but not unilateral tendinopathies, single or multiple injuries in chronic Achilles tendinopathy.

As previously mentioned, these associations were in agreement with previously published findings, where the *VEGFA* rs699947 CC genotype and the A-G-G inferred haplotype constructed from the *VEGFA* rs699947, rs1570360, and rs2010963 variants were associated with increased risk of chronic Achilles tendinopathy in a smaller South African cohort (50). Interestingly, 37.0% (34 of 92) of the participants reported unilateral tendinopathies, whereas 26.8% (19 of 71) reported bilateral tendinopathies in the first and second published South African cohorts respectively (24). The presence of a larger cohort in this study, specifically participants presenting with more bilateral tendinopathies in the second published cohort (24) is one of the possible explanations as to why the *VEGFA* genotype and inferred haplotypes were associated with chronic Achilles tendinopathy in the original study but not the combined analysis presented in this dissertation.

Table 5.1: The association of *VEGFA* and *KDR* variants in this study as well as previously associated variants associated with chronic Achilles Tendinopathy

		SA Rahim (2016)	British Rahim (2016)	Brazilian Salles (2016)	SA Dissertation
***VEGFA* Polymorphism**					
(A) rs699947	-2578 C>A	C-C TEN	not associated	not associated	C-C bilateral TEN
(B) rs1570360	-1154 G>A	not associated	not associated	n.d.	n.d.
(C) rs2010963	-634 G>C	not associated	not associated	n.d.	not associated
(D) rs833061	-460 T>C	n.d.	n.d.	not associated	n.d.
(E) rs3025039	935 C>T	n.d.	n.d.	not associated	n.d.
***VEGFA* A + B + C Hap**		A-G-G TEN	A+B+C Hap not associated	n.d.	A-G bilateral TEN
***VEGFA* A + D + E Hap**		n.d.	n.d.	not associated	n.d.
***KDR* Polymorphism**					
(1) rs2071559	-604 G>A	not associated	not associated	not associated	A-A multiple TEN
(2) rs2305948	1192 G>A	n.d.	n.d.	G-G Tendinopathy	n.d.
(3) rs1870377	1719 T>A	not associated	not associated	not associated	not associated
KDR 1 + 2 + 3 Hap		1+2+3 not associated	1+2+3 not associated	G-G-A and G-A-T Tendinopathy	G-T bilateral and/or multiple TEN A-A bilateral and/or multiple TEN
A + 3 Allele Interactions		n.d.	n.d.	n.d.	C-G bilateral or multiple TEN A-A bilateral or multiple TEN

The *VEGFA* and *KDR* variants and inferred haplotypes, as well as the cohort, analysed in this dissertation are indicated in grey.

Additional findings of the study were that the *KDR* rs2071559 AA genotype was associated with increased risk of a history of multiple (two or more) chronic Achilles tendinopathies (Table 5.1). The rs2071559 variant, specifically the AA genotype, was however not associated with a history of single, unilateral or bilateral Achilles tendon injuries. There was however a significant linear increase in the AA genotype distribution from the control, single Achilles tendinopathy and multiple Achilles tendinopathy groups. Although the *KDR* rs1870377 variant was not independently associated, the G-T and A-A *KDR* inferred haplotypes constructed from rs2071559 and rs1870377 were associated with decreased and increased risk of multiple and/or bilateral chronic Achilles tendinopathies (Table 5.1).

The final set of findings from the results presented in chapter 3 was that there was a tendency for the *VEGFA* rs699947 and *KDR* rs2071559 allele-allele interactions to be associated with chronic Achilles tendinopathy, with the C-G and A-A interactions being significantly associated with decreased and increased risk of chronic Achilles tendinopathy. These allele- allele interactions were however significantly associated with a history of bilateral or multiple Achilles tendon injuries. Specifically, the C-G and A-A interactions were significantly associated with decreased and increased risk of bilateral or multiple injuries respectively (Table 5.1). The *VEGFA* rs699947 allele and *KDR* rs2071559 allele interactions, were however not associated with either a grade III or grade IV chronic Achilles tendinopathy nor an injury specifically related to the middle-third of the tendon.

Although different, *VEGFA* allele and *KDR* allele interactions have previously been reported to modulate the risk of ACL ruptures. Specifically, the A-G-A-A and A-G-G-A allele interactions constructed from *VEGFA* (rs699947 C/A, rs2010963 G/C) and *KDR* (rs2071559 G/A, rs1870377 T/A) were previously associated with decreased risk of injury (Feldman *et al.*, 2022), highlighting the importance of this pathway in modulating the risk of injury.

The functional *VEGFA* rs699947 (C>A) polymorphism is located within the promoter region at nucleotide -2578 (114) and besides tendinopathy, it has also been associated with other pathologies or injuries, such as diabetic retinopathy (42)coronary heart disease (131,132), and ACL ruptures (51,56). Although not always reported the CC genotype, as reported for bilateral Achilles tendinopathy in this dissertation, is associated with reduced risk of pathology or injury. The C-allele of this variant has been associated with increased *VEGFA* expression and by implication promoting angiogenesis (50,56). Although the CC genotype of this variant was

associated with reduced risk of ACL ruptures among Indian athletes (56), it was not independently associated with ACL ruptures in a combined analysis of multiple populations of European ancestry consisting of 912 cases and 765 controls (51). Contrary to the findings reported in this dissertation, where the *VEGFA* A-G inferred haplotype constructed from rs699947 and rs2010963 was associated with bilateral Achilles tendinopathy, the A-A-G and A-G-G inferred haplotypes constructed from rs699947, rs1570360 and rs2010963 were associated with reduced risk of ACL ruptures in the multiple population combined analysis. The rs2010963 CC genotype was also independently associated with increased risk of ACL ruptures in the combined analysis (51). The explanation for these associations with regards to musculoskeletal soft tissue injuries is currently unknown. These associations nevertheless point to the importance of this genetic region, specifically the *VEGFA* gene, in modulating the risk of injury. Therefore, future work is required to identify the responsible variants within this gene.

Although the role of regulatory regions with the gene is not fully understood, *VEGFA* expression, which is upregulated by mechanical loading, hypoxic conditions, inflammatory cytokines and nerve signals, remains an important mechanism in extracellular matrix homeostasis (35,133,134). Healthy adult tendons express negligible amounts of *VEGF*, which increases significantly during remodelling of injured tissues and during wound healing (29,35). An increase in *VEGF*, specifically *VEGFA* expression has been reported in tenocytes (45,135); and in individuals undergoing recovery in the mid-portion of the Achilles tendon (11). The expression of *VEGF* in degenerative and foetal tendons are similar, thereby illustrating its role in healing (118,134).

The functional *KDR* rs2071559 (G>A) polymorphism has been associated with atherosclerotic cardiovascular diseases (119) and age-related macular degeneration (118). As in this dissertation, where the AA genotype was independently associated with increased risk of multiple Achilles tendinopathy, it has also been associated with age-related macular degeneration and peritoneal baseline transport. This *KDR* variant was however not independently associated with ACL ruptures in a combined analysis of multiple populations of European ancestry (51). The rs2071559 A-allele at nucleotide position -604 of the *KDR* promoter is associated with increased transcription (117,120) resulting in the upregulation of *KDR*, *VEGFA* and angiogenesis signalling cascades (136). Further research is required to investigate the deregulatory role of *KDR* in the aetiology of Achilles tendinopathy and other musculoskeletal soft tissue injuries.

The results of this dissertation should be repeated in larger cohorts, not only of European ancestry, but other population groups, where the severity of the Achilles tendinopathy has been well documented. Other variants within the *VEGFA* and *KDR* genes should be included to further define the functional regions within these genes. Future work should investigate the association of *VEGFA* and *KDR* variants with respect to grade I and II injuries, other regions of the Achilles tendon and other tendinopathies.

One of the limitations of the study presented in Chapter 3 was matching participants for confounders, specifically that of age and weight. The Achilles tendinopathy group was older and heavier than the controls. Although the groups were matched for age and weight in the second published cohort (24) the controls were significantly younger and lighter in the first published cohort (50).

5.3 *VEGFA* and *KDR* polymorphisms are not associated with ultrasound and multidimensional pain measurements in participants with chronic Achilles tendinopathy

A cross-sectional genetic association study approach was employed in Chapter 4 of this dissertation to investigate the association of *VEGF* and *KDR* polymorphisms with ultrasound and multidimensional pain measurements in participants with chronic Achilles tendinopathy. The main findings after ultrasound examination of the tendons were that there were no significant differences in the diameters of injured and uninjured Achilles tendons between the *VEGFA* rs699947, *VEGFA* rs2010963 and *KDR* rs1870377 genotype groups, as well asthe *KDR* rs2071559 genotype groups after adjusting for weight. Similarly, there were no significant differences in the relative number of abnormal ultrasound findings of injured and uninjured tendons between the *VEGFA* rs699947, *VEGFA* rs2010963 and *KDR* rs1870377 variants, as well as *KDR* rs2071559 after adjusting for weight.

Although abnormal ultrasound findings, which included thicker tendons, abnormal tendon ultrasound findings and the presence of neovascularisation, were previously described in this cohort, the *VEGFA* and *KDR* genotype effects on neovascularisation could not be determined due to the small number of participants with neovascularisation in this cohort (24). High levels of neovascularisation associated with Achilles tendinopathy were not constant findings, with as little a 4% and as high as 97% being reported in studies investigating ruptured Achilles tendons

(24,135), rotator cuff tendinopathies (119) and ligament injuries (37,137). Although not consistently reported, some authors have reported the risk of developing Achilles tendinopathy can be as high as seven-fold in the presence of neovascularisation (138) The neovascularisation hypothesis however remains one of the possible mechanisms for tendon pain due to the anatomical interactions between nerve fibres and blood vessels in Achilles tendinopathy(3). The presence of neovascularisation in the Achilles tendon indicates hypoxic conditions and compromised tissue repair, which is associated with pain (38–41,43). Although regulated by many factors including mechanical loading, as previously mentioned, *VEGFA* and *KDR* are key regulators of neovascularisation and therefore, further work should investigate the interaction of these genes and their regulatory roles in neovascularisation (33,44).

The main findings from the multidimensional pain measurements were that there were no significant differences in the Victorian Institute Sports Assessment – Achilles (VISA-A), short-form McGill Pain Questionnaire (sf-MPQ) and short-form Brief Pain Inventory (sf-BPI), as well the subscales and scores between the *VEGFA* rs699947, *VEGFA* rs2010963, *KDR* rs2071559 and *KDR* rs1870377 genotype groups when appropriately adjusted for sex. This was required since females reported higher sf-BPI totals and intensity sub-scale scores compared to their male counterparts (24).

Achilles tendon pain was previously described in this cohort as predominately a sensory type of pain with the four descriptors of the affective type of pain, namely sickening, punishing- cruel, fearful and tiring-exhausting were rarely used (24). The participants predominately used descriptors of continuous sensory pain, namely aching, tender, throbbing, and less frequently as descriptors of intermittent or neuropathic sensory pain, which further affects the mood, walking ability, sleep and enjoyment of life among those affected (24). The *COMT* rs4818 CCgenotype was previously reported to be associated with higher self-reported tendon pain scoreswhen using the sf-MPQ, suggesting that the catecholaminergic pathway is involved in the painassociated with chronic Achilles tendinopathy. No other associations were reported of the *TAC1*rs2072100, *TACR1* rs3771829 and *SCN9A* rs746030 polymorphisms involved in pain pathways. None of these variants were associated with the total pain scores of the sf-BPI, sf- MPQ and VISA-A.

Since the *KDR* receptor permits the entry of cations through a non-selective membrane known as the transient receptor channel which regulates the pain experienced among individuals (49,104,139–141) the involvement of the *VEGF-KDR* pathway in the aetiology of the pain

associated with chronic Achilles tendinopathy therefore warrants further research (139–141). Future research should also consider that pain is a complex symptom which also consists of gender, ethnic and epigenetic components (140,141). Many of the participants included in this study continued running regardless of the pain in the Achilles tendon (grades I to III). Therefore, future research should specifically recruit participants whose pain is so severe that they stop training (grade IV).

5.4 Conclusion

In conclusion, the *VEGFA* rs699947 and *KDR* rs2071559 polymorphisms were associated with bilateral tendinopathies and multiple injuries to the Achilles tendon. The *VEGFA* rs699947 C- allele and *KDR* rs2071559 G-allele decreased, whereas the *VEGFA* rs699947 A-allele and *KDR*rs2071559 A-allele increased the risk of chronic bilateral tendinopathies and multiple injuries to the Achilles tendon. This study was the first to specifically investigate the association of *VEGF* and *KDR* on chronic bilateral Achilles tendinopathies and multiple injuries. Future studies should further explore these associations as well as other variants within these polymorphisms and their associations with chronic bilateral Achilles tendinopathies and multiple injuries.

References

1. O'Brien M. Functional anatomy and physiology of tendons. Clin Sports Med. 1992;11(3):505–20.
2. Rufai A, Ralphs JR, Benjamin M. Structure and histopathology of the insertional region of the human achilles tendon. Journal of Orthopaedic Research. 1995 Jul 18;13(4):585–93.
3. Li HY, Hua YH. Achilles Tendinopathy: Current Concepts about the Basic Science and Clinical Treatments. Biomed Res Int. 2016;2016:1–9.
4. Doral MN, Alam M, Bozkurt M, Turhan E, Atay OA, Dönmez G, et al. Functional anatomy of the Achilles tendon. Knee Surgery, Sports Traumatology, Arthroscopy. 2010;18(5):638–43.
5. Abate M, Gravare Silbernagel K, Siljeholm C, Di Iorio A, De Amicis D, Salini V, et al. Pathogenesis of tendinopathies: inflammation or degeneration? Vol. 11, Arthritis research & therapy. 2009. p. 235.
6. McShane JM, Ostick B, McCabe F. Noninsertional Achilles tendinopathy: Pathology and management. Curr Sports Med Rep. 2007 Oct 5;6(5):288–92.
7. Ahmed IM, Lagopoulos M, Soames RW, Sefton GK., Mcconnell P. Blood supply of the Achilles tendon.. 1998;16(5):591-596. J Orthop Res. 1998;16(5):591–6.
8. Steinmann S, Pfeifer CG, Brochhausen C, Docheva D. Spectrum of tendon pathologies: Triggers, trails and end-state. Vol. 21, International Journal of Molecular Sciences. MDPI AG; 2020.
9. Rabin A, Kozol Z, Finestone AS. Limited ankle dorsiflexion increases the risk for mid-portion Achilles tendinopathy in infantry recruits: A prospective cohort study. J Foot Ankle Res. 2014;7(1).
10. Salles JI, Duarte MEL, Guimarães JM, Lopes LR, Vilarinho Cardoso J, Aguiar DP, et al. Vascular Endothelial Growth Factor Receptor-2 Polymorphisms Have Protective Effect against the Development of Tendinopathy in Volleyball Athletes. PLoS One. 2016 Dec 8;11(12):e0167717.
11. Vasta S, Di Martino A, Zampogna B, Torre G, Papalia R, Denaro V. Role of VEGF, nitric oxide, and sympathetic neurotransmitters in the pathogenesis of tendinopathy: A review of the current evidence. Vol. 8, Frontiers in Aging Neuroscience. Frontiers Media S.A.; 2016.
12. Albano D, Messina C, Usuelli FG, De Girolamo L, Grassi M, Maccario C, et al. Magnetic resonance and ultrasound in achilles tendinopathy: Predictive role and response assessment to platelet-rich plasma and adipose-derived stromal vascular fraction injection. Eur J Radiol. 2017 Oct 1;95:130–5.
13. Astrom M. On the nature and etiology of chronic achilles tendinopathy. . Clin Orthop Relat Res. 1995;
14. Lagas IF, Fokkema T, Verhaar JAN, Bierma-Zeinstra SMA, van Middelkoop M, de Vos RJ. Incidence of Achilles tendinopathy and associated risk factors in recreational runners: A large prospective cohort study. J Sci Med Sport. 2020 May 1;23(5):448–52.
15. Raja SN, Carr DB, Cohen M, Finnerup NB, Flor H, Gibson S, et al. The revised International Association for the Study of Pain definition of pain: concepts, challenges, and compromises. Pain. 2020 Sep;161(9):1976–82.
16. 130. Plinsinga M, BM, VB, & van WC. Evidence of Nervous System Sensitization in Commonly Presenting and Persistent Painful Tendinopathies: A Systematic Review. J Orthop Sport Phys Ther. 2015;45:864–75.

17. Tompra N, van Dieën JH, Coppieters MW. Central pain processing is altered in people with Achilles tendinopathy. Br J Sports Med. 2016 Aug;50(16):1004–7.

18. Cook JL, Khan KM, Purdam C. Achilles tendinopathy. Man Ther. 2002;7(3):121–30.

19. 99. Lian Ø, DJ, AP, FF, EL, & BR. Pronociceptive and antinociceptive neuromediators in patellar tendinopathy. Am J Sports Me. 2006;34:1801–8.

20. Schizas N, Weiss R, Lian O, Frihagen F, Bahr R, Ackermann PW. Glutamate receptors in tendinopathic patients. Journal of Orthopaedic Research. 2012 Sep;30(9):1447–52.

21. Bakkegaard M, JFE, HB, & LH. Ultrasonography as a prognostic and objective parameter in Achilles tendinopathy: a prospective observational study. uropean journal of radiology. 2015;

22. Robinson JM, Cook JL, Purdam C, Visentini PJ, Ross J, Mavulli N, et al. The VISA-A questionnaire: a valid and reliable index of the clinical severity of Achilles tendinopathy [Internet]. Available from: http://bjsm.bmjjournals/cgi/content/full/35/5/336/DC1.

23. Mkumbuzi NS, September A V., Posthumus M, Oulo B, Mafu TS, Collins M. Characterisation of Achilles tendon pain in recreational runners using multidimensional pain scales. J Sci Med Sport. 2020 Mar 1;23(3):258–63.

24. Mkumbuzi NS, Jørgensen OH, Mafu TS, September A V., Posthumus M, Collins M. Ultrasound findings are not associated with tendon pain in recreational athletes with chronic Achilles tendinopathy. Transl Sports Med. 2020 Nov 1;3(6):589–98.

25. Boyle GJ, Boerresen BH, Jang DM. Factor analyses of the mcgill pain questionnaire (MPQ) in acute and chronic pain patients. Psychol Rep. 2015 Jun 1;116(3):797–820.

26. Melzack R. The short-form McGill Pain Questionnaire. Pain. 1987;30(2):191–7.

27. Jumbo SU, MacDermid JC, Kalu ME, Packham TL, Athwal GS, Faber KJ. Measurement properties of the brief pain inventory- short form (BPI-SF) and the revised short mcgill pain questionnaire-version-2 (SF-MPQ-2) in pain-related musculoskeletal conditions: A systematic review protocol. Vol. 8, Archives of Bone and Joint Surgery. Mashhad University of Medical Sciences; 2020. p. 131–41.

28. Mkumbuzi NS, Mafu TS, September A V., Posthumus M, Collins M. Conditioned pain modulation is not altered in recreational athletes with Achilles tendinopathy. Transl Sports Med. 2021 Jan 1;4(1):147–53.

29. Mousavizadeh R, Khosravi S, Behzad H, McCormack RG, Duronio V, Scott A. Cyclic strain alters the expression and release of angiogenic factors by human tendon cells. PLoS One. 2014 May 13;9(5).

30. Battery L &Maffulli, N. Inflammation in overuse tendon injuries. Sports Med Arthrosc, . 2011;19:213–7.

31. Dean BJF, Snelling SJB, Dakin SG, Murphy RJ, Javaid MK, Carr AJ. Differences in glutamate receptors and inflammatory cell numbers are associated with the resolution of pain in human rotator cuff tendinopathy. Arthritis Res Ther. 2015 Jul 10;17(1).

32. Rio E, Moseley L, Purdam C, Samiric T, Kidgell D, Pearce AJ, et al. The pain of tendinopathy: Physiological or pathophysiological? Vol. 44, Sports Medicine. 2014. p. 9–23.

33. Szwedowski D, Jaworski Ł, Szwedowska W, Pękala P, Gagat M. Neovascularisation in meniscus and tendon pathology as a potential mechanism in regenerative therapies: Special reference to platelet-rich plasma treatment. Vol. 11, Applied Sciences (Switzerland). MDPI; 2021.

34. Aström M& RA. Chronic Achilles tendinopathy. A survey of surgical and histopathologic findings. Clin Orthop Relat Res. 1995;

35. Petersen W, Pufe T, Kurz B, Mentlein R, Tillmann B. Angiogenesis in fetal tendon development: Spatial and temporal expression of the angiogenic peptide vascular endothelial cell growth factor. Anat Embryol (Berl). 2002;205(4):263–70.

36. Lakemeier S, Reichelt JJ, Patzer T, Fuchs-Winkelmann S, Paletta JR, Schofer MD. The association between retraction of the torn rotator cuff and increasing expression of hypoxia inducible factor 1 and vascular endothelial growth factor expression: An immunohistological study. BMC Musculoskelet Disord. 2010;11.

37. Beyer R, Kongsgaard M, Hougs Kjær B, Øhlenschlæger T, Kjær M, Magnusson SP. Heavy slow resistance versus eccentric training as treatment for achilles tendinopathy: A randomized controlled trial. American Journal of Sports Medicine. 2015 Jul 3;43(7):1704–11.

38. Cook JL, Malliaras P, De Luca J, Ptasznik R, Morris ME, Goldie P. Neovascularisation and Pain in Abnormal Patellar Tendons of Active Jumping Athletes [Internet]. 2004. Available from: http://journals.lww.com/cjsportsmed

39. Foulkes T, Wood JN. Pain genes. Vol. 4, PLoS Genetics. 2008.

40. Järvinen TAH. Neovascularisation in tendinopathy: from eradication to stabilisation? Vol. 54, British Journal of Sports Medicine. BMJ Publishing Group; 2020. p. 1–2.

41. Reiter M, Ulreich N, Dirisamer A, Bucek R. Colour and power Doppler sonography in symptomatic Achilles tendon disease. . Int J Sports Med. 2004;25(4):301–5.

42. Liu X, Zhu B, Li Y, Liu X, Guo S, Wang C, et al. The Role of Vascular Endothelial Growth Factor in Tendon Healing. Front Physiol. 2021 Oct 25;12.

43. Tol JL, Spiezia F, Maffulli N. Neovascularisation in Achilles tendinopathy: Have we been chasing a red herring? Vol. 20, Knee Surgery, Sports Traumatology, Arthroscopy. Springer Verlag; 2012. p. 1891–4.

44. Hulse RP. Role of VEGF-A in chronic pain [Internet]. Vol. 8, Oncotarget. 2017. Available from: www.impactjournals.com/oncotarget/

45. Hulse RP, Beazley-Long N, Hua J, Kennedy H, Prager J, Bevan H, et al. Regulation of alternative VEGF-A mRNA splicing is a therapeutic target for analgesia. Neurobiol Dis. 2014 Nov 1;71:245–59.

46. Maffulli N, Sharma P, Luscombe KL. Achilles tendinopathy: aetiology and management. JRSM. 2004 Oct 1;97(10):472–6.

47. Gil-Melgosa L, Grasa J, Urbiola A, Llombart R, Ruiz MS, Montiel V, et al. Muscular and tendon degeneration after achilles rupture: New insights into future repair strategies. Biomedicines. 2022 Jan 1;10(1).

48. Sánchez-Sánchez JL, Calderón-Díez L, Herrero-Turrión J, Méndez-Sánchez R, Arias-Buría JL, Fernández-de-las-Peñas C. Changes in Gene Expression Associated with Collagen Regeneration and Remodeling of Extracellular Matrix after Percutaneous Electrolysis on Collagenase-Induced Achilles Tendinopathy in an Experimental Animal Model: A Pilot Study. J Clin Med. 2020 Oct 15;9(10):3316.

49. Llorián-Salvador M, González-Rodríguez S. Painful Understanding of VEGF. Front Pharmacol. 2018 Nov 6;9.

50. Rahim M, El Khoury LY, Raleigh SM, Ribbans WJ, Posthumus M, Collins M, et al. Human Genetic Variation, Sport and Exercise Medicine, and Achilles Tendinopathy: Role for Angiogenesis-Associated Genes. OMICS. 2016 Sep 1;20(9):520–7.

51. Feldmann DC, Rahim M, Suijkerbuijk MAM, Laguette MJN, Cieszczyk P, Ficek K, et al. Investigation of multiple populations highlight VEGFA polymorphisms to modulate anterior cruciate ligament injury. Journal of Orthopaedic Research. 2022 Jul 1;40(7):1604–12.

52. Lulińska-Kuklik E, Leźnicka K, Humińska-Lisowska K, Moska W, Michałowska-Sawczyn M, Ossowski Z, et al. The VEGFA gene and anterior cruciate ligament rupture risk in the Caucasian population. Biol Sport. 2019;36(1):3–8.

53. Rahim M, Hobbs H, van der Merwe W, Posthumus M, Collins M, September A V. Investigation of angiogenesis genes with anterior cruciate ligament rupture risk in a South African population. J Sports Sci. 2018 Mar 4;36(5):551–7.

54. Rahim M, Gibbon A, Hobbs H, Van Der Merwe W, Posthumus M, Collins M, et al. The association of genes involved in the angiogenesis-associated signaling pathway with risk of anterior cruciate ligament rupture. Journal of Orthopaedic Research. 2014 Dec 1;32(12):1612–8.

55. Masouda Rahim MLMCMPAVS. Risk modelling further implicates the angiogenesis pathway in anterior cruciate ligament ruptures. Eur J Sport Sci. 2021;22(4):650–7.

56. Shukla M, Gupta R, Pandey V, Rochette J, Dhandapany PS, Tiwari PK, et al. VEGFA Promoter Polymorphisms rs699947 and rs35569394 Are Associated With the Risk of Anterior Cruciate Ligament Ruptures Among Indian Athletes: A Cross-sectional Study. Orthop J Sports Med. 2020 Dec 1;8(12):232596712096447.

57. Rahim M, Gibbon A, Collins M, September A V. Genetics of musculoskeletal soft tissue injuries: Current status, challenges, and future directions. In: Sports, Exercise, and Nutritional Genomics: Current Status and Future Directions. Elsevier; 2019. p. 317–39.

58. Ribbans WJ, September A V., Collins M. Tendon and Ligament Genetics: How Do They Contribute to Disease and Injury? A Narrative Review. Life. 2022 Apr 29;12(5):663.

59. O'Brien M. The Anatomy of the Achilles Tendon. Foot Ankle Clin. 2005 Jun;10(2):225–38.

60. Kader D. Achilles tendinopathy: some aspects of basic science and clinical management [Internet]. Vol. 36, Br J Sports Med. 2002. Available from: www.bjsportmed.com

61. Theobald P, Bydder G, Dent C, Nokes L, Pugh N, Benjamin M. The functional anatomy of Kager's fat pad in relation to retrocalcaneal problems and other hindfoot disorders. J Anat. 2006 Jan;208(1):91–7.

62. Benjamin M, McGonagle D. The anatomical basis for disease localisation in seronegative spondyloarthropathy at entheses and related sites. Vol. 199, Journal of Anatomy. Cambridge University Press; 2001. p. 503–26.

63. M. Benjamin HTJRRGBTMBSM. Where tendons and ligaments meet bone: attachment sites ('entheses') in relation to exercise and/or mechanical load. J Anat. 2006;208(4).

64. Sharma P, Maffulli N, Maffulli N. Tendon structure Biology of tendon injury: healing, modeling and remodeling. Vol. 6, J Musculoskelet Neuronal Interact. 2006.

65. Ahmed. Blood supply of the Achilles tendon.

66. O'Brien M. Structure and metabolism of tendons. Scand J Med Sci Sports. 1997;7(2):55–61.

67. Kannus P. Structure of the tendon connective tissue. Scand J Med Sci Sports. 2008;

68. Winnicki K, Ochała-Kłos A, Rutowicz B, Pękala PA, Tomaszewski KA. Functional anatomy, histology and biomechanics of the human Achilles tendon — A comprehensive review. Vol. 229, Annals of Anatomy. Elsevier GmbH; 2020.

69. Ribbans W, Collins M. Pathology of the tendo Achillis: do our genes contribute? . The Journal of Bone and Joint Surgery B . 2013;95(3):305–13.

70. Tresoldi I, Oliva F, Benvenuto M, Fantini M, Masuelli L, Bei R, et al. Muscles, Ligaments and Tendons Journal 2013; 3 (1): 2-6 Tendon's ultrastructure.

71. Kirkendall D. T. GWE. Function and biomechanics of tendons. Scand J Med Sci Sports. 1997;7(2):62–6.

72. Ippolito E. NPG, PF, AL, DMC. Morphological, immunochemical, and biochemical study of rabbit Achilles tendon at various ages. J Bone Joint Surg. 1980;65(4):583–98.

73. Roche AJ, Calder JDF. Achilles tendinopathy. Bone Joint J. 2013 Oct;95-B(10):1299–307.

74. Binfield PM & Maffulli N. Surgical management of common tendinopathies of the lower limb. . Sports Exercise Injuries . 1997;3:116–22.

75. Roughley PJ, Mort JS. The role of aggrecan in normal and osteoarthritic cartilage. J Exp Orthop. 2014 Dec 16;1(1):8.

76. Sobhani S, Dekker R, Postema K, Dijkstra PU. Epidemiology of ankle and foot overuse injuries in sports: A systematic review. Vol. 23, Scandinavian Journal of Medicine and Science in Sports. 2013. p. 669–86.

77. Lysholm J, Wiklander J. Injuries in runners. Am J Sports Med. 1987 Mar 23;15(2):168–71.

78. Fredberg U, Stengaard-Pedersen K. Chronic tendinopathy tissue pathology, pain mechanisms, and etiology with a special focus on inflammation: Review. Scand J Med Sci Sports. 2008 Feb;18(1):3–15.

79. James SL BBOLR. Injuries to runners. Am J Sports Med . 1978;6:40–50.

80. Chad A, Thomas M. Achilles tendon disorders. BMJ 2013;346:1–7. . BMJ. 2013;346:1–7.

81. Kountouris A CJ. Rehabilitation of Achilles and patellar tendinopathies. . . Best Pract Res Clin Rheumatol . 2007;21:295–316.

82. 68. Hutchison AM ERBOPITCWPVNMVBD. What is the best clinical test for achilles tendinopathy? . Foot Ankle Surg . 2013;19:112–7.

83. Khan KM, Cook JL, Bonar F, Harcourt P, Åstrom M. Histopathology of Common Tendinopathies Update and Implications for Clinical Management.

84. Budnick A, Kuhnert R, Könner F, Kalinowski S, Kreutz R, Dräger D. Validation of a modified German version of the brief pain inventory for use in nursing home residents with chronic pain. Journal of Pain. 2016 Feb 1;17(2):248–56.

85. 28. Celik EC YEAFKMOKSTEIPNGIMTCCS. Validity and reliability of a Turkish Brief Pain Inventory Short Form when used to evaluate musculoskeletal pain. J Back Musculoskelet Rehabil . 2017;30:229–33.

86. 126. Parker R JJSD. Pain in amaXhosa women living with HIV/AIDS: Translation and validation of the brief pain inventory-Xhosa. J Pain Symptom Manage . 2016;51:126–32.

87. Dakin SG, Buckley CD, Al-Mossawi MH, Hedley R, Martinez FO, Wheway K, et al. Persistent stromal fibroblast activation is present in chronic tendinopathy. Arthritis Res Ther. 2017 Jan 25;19(1).

88. Trojian T, Amoako A. Tendinopathy Not Tendonitis Now Is the Time for a Change [Internet]. 2015. Available from: www.acsm-healthfitness.org

89. Alfredson. Glutamate NMDAR1 receptors localised to nerves in human Achilles tendons. Implications for treatment? H Alfredson 1, S Forsgren, K Thorsen, M Fahlström, H Johansson, R Lorentzon. Knee Surg Sports Traumatol Arthrosc. 2001;

90. Schmid-Schönbein GW. ANALYSIS OF INFLAMMATION. Annu Rev Biomed Eng. 2006 Aug 1;8(1):93–151.

91. Sharma P, Maffulli N. Tendon injury and tendinopathy: Healing and repair. Vol. 87, Journal of Bone and Joint Surgery. Journal of Bone and Joint Surgery Inc.; 2005. p. 187–202.

92. Alberts B JALJ et al. Ion Channels and the Electrical Properties of Membranes. . Garland Science. 2002;

93. Irwin TA. Current concepts review: Insertional Achilles tendinopathy. Foot Ankle Int. 2010 Oct;31(10):933–9.

94. Magra M, Hughes S, El Haj AJ, Maffulli N. VOCCs and TREK-1 ion channel expression in human tenocytes. Am J Physiol Cell Physiol. 2007 Mar;292(3).

95. Cortright DW, Szallasi A. Biochemical pharmacology of the vanilloid receptor TRPV1: An update. Vol. 271, European Journal of Biochemistry. 2004. p. 1814–9.

96. L J Backman GAGFHAASPD. Alpha-2 adrenergic stimulation triggers Achilles tenocyte hypercellularity: Comparison between two model systems. Scand J Med Sci Sports . 2013;

97. Kellenberger S, Schild L. Epithelial Sodium Channel/Degenerin Family of Ion Channels: A Variety of Functions for a Shared Structure. 2002; Available from: www.prv.org

98. Woolf CJ. Neuronal plasticity: increasing the gainin pain. Science (1979). 2000;288:1765–9.

99. Mccleskey EW, Gold MS. ION CHANNELS OF NOCICEPTION [Internet]. Vol. 61, Annu. Rev. Physiol. 1999. Available from: www.annualreviews.org

100. Johnson JW, Ascher P. Voltage-dependent block by intracellular Mg2+ of N-methyl-D-aspartate-activated channels.

101. Zellers JA, Cortes DH, Pohlig RT, Silbernagel KG. Tendon morphology and mechanical properties assessed by ultrasound show change early in recovery and potential prognostic ability for 6-month outcomes. Knee Surgery, Sports Traumatology, Arthroscopy. 2019 Sep 1;27(9):2831–9.

102. Sharma P, Maffulli N. Understanding and managing Achilles tendinopathy. Br J Hosp Med. 2006 Feb;67(2):64–7.

103. Sharma P, Maffulli N. Tendinopathy and tendon injury: The future. Disabil Rehabil. 2008;30(20–22):1733–45.

104. Lee GW, Son JY, Lee AR, Ju JS, Bae YC, Ahn DK. Central VEGF-A pathway plays a key role in the development of trigeminal neuropathic pain in rats. Mol Pain. 2019 Jan 30;15:174480691987260.

105. Qian Y, Ding L, Cao L, Yu Z, Shao X, Wang L, et al. Gene polymorphisms of VEGF and KDR are associated with initial fast peritoneal solute transfer rate in peritoneal dialysis. BMC Nephrol. 2022 Nov 14;23(1):365.

106. Petersen W, Pufe T, Unterhauser F, Zantop T, Mentlein R, Weiler A. The splice variants 120 and 164 of the angiogenic peptide vascular endothelial cell growth factor (VEGF) are expressed during Achilles tendon healing. Arch Orthop Trauma Surg. 2003 Nov 1;123(9):475–80.

107. Zhang H, Faber JE. Trophic effect of norepinephrine on arterial intima-media and adventitia is augmented by injury and mediated by different α1-adrenoceptor subtypes. Circ Res. 2001 Oct 26;89(9):815–22.

108. Hulse RP, Drake RAR, Bates DO, Donaldson LF. The control of alternative splicing by SRSF1 in myelinated afferents contributes to the development of neuropathic pain. Neurobiol Dis. 2016 Dec 1;96:186–200.

109. Corrêa JB, Costa LOP, de Oliveira NTB, Sluka KA, Liebano RE. Central sensitization and changes in conditioned pain modulation in people with chronic nonspecific low back pain: a case–control study. Exp Brain Res. 2015 Aug 25;233(8):2391–9.

110. Kiguchi N, Kobayashi Y, Kadowaki Y, Fukazawa Y, Saika F, Kishioka S. Vascular endothelial growth factor signaling in injured nerves underlies peripheral sensitization in neuropathic pain. J Neurochem. 2014;129(1):169–78.

111. Öhberg L, Alfredson H. Ultrasound guided sclerosis of neovessels in painful chronic Achilles tendinosis: Pilot study of a new treatment. Br J Sports Med. 2002;36(3):173–5.

112. Alfredson H, Lorentzon M, Bäckman S, Bäckman A, Lerner UH. cDNA-arrays and real-time quantitative PCR techniques in the investigation of chronic achilles tendinosis. Journal of Orthopaedic Research. 2003;21(6):970–5.

113. Vincenti V, CC, RM, & PG. Assignment of the vascular endothelial growth factor gene to human chromosome . Circulation. 1996;93(8):1493–5.

114. Tischer E, Mitchell$ R, Hartman T, Silva M, Gospodarowiczs D, Fiddes JC, et al. THE JOURNAL OF BIOLOGICAL CHEMISTRY The Human Gene for Vascular Endothelial Growth Factor MULTIPLE PROTEIN FORMS ARE ENCODED THROUGH ALTERNATIVE EXON SPLICING*. Vol. 266. 1991.

115. Shahbazi M, Fryer AA, Pravica V, Brogan IJ, Ramsay HM, Hutchinson I V, et al. Vascular Endothelial Growth Factor Gene Polymorphisms Are Associated with Acute Renal Allograft Rejection [Internet]. 2001. Available from: http://journals.lww.com/jasn

116. 94. Lambrechts D SEMMDFJDFMS et al. VEGF is a modifier of amyotrophic lateral sclerosis in mice and humans and protects motoneurons against ischemic death. Nat Genet. 2003;34:384–94.

117. Fraga A, Ribeiro R, Coelho A, Vizcaíno JR, Coutinho H, Lopes JM, et al. Genetic polymorphisms in key hypoxia-regulated downstream molecules and phenotypic correlation in prostate cancer. BMC Urol. 2017 Jan 31;17(1):1–12.

118. Galan A, Ferlin A, Caretti L, Buson G, Sato G, Frigo AC, et al. Association of age-related macular degeneration with polymorphisms in vascular endothelial growth factor and its receptor. Ophthalmology. 2010 Sep;117(9):1769–74.

119. Wang L, Ge H, Peng L, Wang B. A meta-analysis of the relationship between VEGFR2 polymorphisms and atherosclerotic cardiovascular diseases. Clin Cardiol. 2019 Oct 1;42(10):860–5.

120. Wang Y, Zheng Y, Zhang W, Yu H, Lou K, Zhang Y, et al. Polymorphisms of KDR Gene Are Associated With Coronary Heart Disease. J Am Coll Cardiol. 2007 Aug 21;50(8):760–7.

121. Cuschieri S. The STROBE guidelines. Vol. 13, Saudi Journal of Anaesthesia. Wolters Kluwer Medknow Publications; 2019. p. S31–4.

122. Little J, Higgins JPT, Ioannidis JPA, Moher D, Gagnon F, von Elm E, et al. STrengthening the REporting of Genetic Association Studies (STREGA)— An Extension of the STROBE Statement. PLoS Med. 2009 Feb 3;6(2):e1000022.

123. Regulatory Action and News Helsinki Declaration: 2013 revision [Internet]. Available from: http://www.wma.

124. Peers KHE, Brys PPM, Lysens RJJ. Correlation between power Doppler ultrasonography and clinical severity in Achilles tendinopathy. Int Orthop. 2003;27(3):180–3.

125. Lahiri DK, Nurnberger JI. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Vol. 19, Nucleic Acids Research. 1973.

126. Mokone GG, Gajjar M, September A V., Schwellnus MP, Greenberg J, Noakes TD, et al. The Guanine-Thymine Dinucleotide Repeat Polymorphism within the Tenascin-C Gene is Associated with Achilles Tendon Injuries. Am J Sports Med. 2005 Jul 30;33(7):1016–21.

127. Crossley BM, Bai J, Glaser A, Maes R, Porter E, Killian ML, et al. Guidelines for Sanger sequencing and molecular assay monitoring. Journal of Veterinary Diagnostic Investigation. 2020 Nov 1;32(6):767–75.

128. Warnes G, Gorjanc G, Leisch F, Man M. Genetics: Population Genetics. R package version 1381. 2012;

129. González JR, Armengol L, Solé X, Guinó E, Mercader JM, Estivill X, et al. SNPassoc: an R package to perform whole genome association studies. Bioinformatics. 2007 Mar 1;23(5):654–5.

130. Sinnwell J, Schaid D. Statistical Analysis of Haplotypes with Traits and Covariates when Linkage Phase is Ambiguous. 2018.

131. Hajer F, Hana S, Saoussen C, Abdelhak F, Nadia B, Ameni D, et al. Genetic polymorphisms in VEGFA and VEGFR2 genes associated with coronary heart disease susceptibility and severity. Mol Biol Rep. 2023 Dec 1;50(12):10169–77.

132. Wang Y, Huang Q, Liu J, Wang Y, Zheng G, Lin L, et al. Vascular endothelial growth factor A polymorphisms are associated with increased risk of coronary heart disease: a meta-analysis [Internet]. Vol. 8, Oncotarget. 2017. Available from: www.impactjournals.com/oncotarget/

133. Neufeld G, Cohen T. Vascular endothelial growth factor (VEGF) and its receptors. FASEB journal : official publication of the Federation of American Societies for Experimental Biology,. 1999;13(1):9–22.

134. Legerlotz K, Marzilger R, Bohm S, Arampatzis A. Physiological Adaptations following Resistance Training in Youth Athletes—A Narrative Review. Pediatr Exerc Sci. 2016 Nov;28(4):501–20.

135. Mehdizadeh A, Gardiner BS, Lavagnino M, Smith DW. Predicting tenocyte expression profiles and average molecular concentrations in Achilles tendon ECM from tissue strain and fiber damage. Biomech Model Mechanobiol. 2017 Aug 13;16(4):1329–48.

136. Hansen TF, Sørensen FB, Spindler KLG, Olsen DA, Andersen RF, Lindebjerg J, et al. Microvessel density and the association with single nucleotide polymorphisms of the vascular endothelial growth factor receptor 2 in patients with colorectal cancer. Virchows Archiv. 2010 Mar;456(3):251–60.

137. Yang X, Pugh ND, Coleman DP, Nokes LDM. Are Doppler studies a useful method of assessing neovascularisation in human Achilles tendinopathy? A systematic review and suggestions for optimizing machine settings. Vol. 34, Journal of Medical Engineering and Technology. 2010. p. 365–72.

138. Hirschmüller A, Frey V, Konstantinidis L, Baur H, Dickhuth HH, Südkamp NP, et al. Prognostic value of achilles tendon doppler sonography in asymptomatic runners. Med Sci Sports Exerc. 2012 Feb;44(2):199–205.

139. Webborn ADJ. Novel approaches to tendinopathy. Disabil Rehabil. 2008;30(20–22):1572–7.

140. Tan EC, Lim ECP, Ocampo CE, Allen JC, Sng BL, Sia AT. Common variants of catechol-O-methyltransferase influence patient-controlled analgesia usage and postoperative pain in patients undergoing total hysterectomy. Pharmacogenomics Journal. 2016 Apr 1;16(2):186–92.

141. Stephens KE, Levine JD, Aouizerat BE, Paul SM, Abrams G, Conley YP, et al. Associations between genetic and epigenetic variations in cytokine genes and mild persistent breast pain in women following breast cancer surgery. Cytokine. 2017 Nov 1;99:203–13.

Appendix: Supplementary Tables

Supplementary Table S3.1: Participant characteristics of the second previously published cohort (24) asymptomatic control (CON) and chronic Achilles Tendinopathy (TEN) groups

	CON (n = 74)	**TEN** (n = 81)	**p-value**
Age (years) [1]	41.0 ± 14.1	42.3 ± 11.6	0.562
Sex (% male)	57.3	57.5	0.988
Height (cm)	174.7 ± 9.6	173.8 ± 8.3	0.547
Weight (kg) [2]	72.3 ± 13.3	74.8 ± 14.1	0.289
BMI ($kg.m^{-2}$) [2]	23.3 ± 3.4	24.5 ± 3.7	0.053

Except for sex, which is expressed as a percentage, values are expressed as mean ± standard deviation

P-values in bold typeset indicates significance ($p<0.05$)

BMI, body mass index; cm, centimetres; kg, kilograms; m, metres

[1]Age for the CON and TEN groups are at the time of recruitment and first injury respectively

[2]Weight and therefore BMI are self-reported values at the time of recruitment for both groups

Supplementary Table S3.2: Participant characteristics of the first previously published cohort's (50) asymptomatic control (CON) and chronic Achilles Tendinopathy (TEN) groups

	CON (n = 120)	**TEN** (n = 104)	**p-value**
Age (years) [1]	37.3 ± 10.4	42.2 ± 13.9	**0.004**
Sex (% male)	64.7	69.5	0.411
Height (cm)	176.4 ± 9.5	176.4 ± 8.7	0.262
Weight (kg) [2]	71.4 ± 12.1	78.8 ± 18.4	**<0.001 (0.001)** [3]
BMI ($kg.m^{-2}$) [2]	23.3 ± 2.7	25.3 ± 4.3	**<0.001 (0.001)** [3]

Except for sex, which is expressed as a percentage, values are expressed as mean ± standard deviation

P-values in bold typeset indicates significance ($p<0.05$)

BMI, body mass index; cm, centimetres; kg, kilograms; m, metres

[1]Age for the CON and TEN groups are at the time of recruitment and first injury respectively

[2]Weight and therefore BMI are self-reported values at the time of recruitment for both groups

[3]Adjusted p-values at the age of recruitment

Supplementary Table S3.3: *VEGFA* rs699947 (-2578 C>A) genotype associations on participant characteristics of the combined asymptomatic control (CON) and chronic Achilles Tendinopathy (TEN) groups of both cohorts.

	CC (n = 92)	**CA** (n = 177)	**AA** (n = 90)	**p-value**
Age (years) [1]	40.5 ± 12.8 (87)	40.6 ± 12.7 (166)	39.9 ± 11.6 (88)	0.913
Sex (% male)	60.9 (56)	58.2 (103)	74.4 (67)	**0.036** [3]
Height (cm)	175.1 ± 9.1 (79)	173.7 ± 8.7 (164)	177.7 ± 9.5 (87)	**0.003 (<0.001)**[4]
Weight (kg) [2]	73.7 ± 13.3 (82)	73.1 ± 13.8 (168)	77.8 ± 18.1 (87)	**0.049** (0.076)[5]
BMI ($kg.m^{-2}$) [2]	23.9 ± 3.4 (83)	24.1 ± 3.5 (168)	24.4 ± 4.2 (86)	0.654

Except for sex, which is expressed as a percentage, values are expressed as mean ± standard deviation

P-values in bold typeset indicates significance ($p<0.05$)

[1]Age for the CON and TEN groups are at the time of recruitment and first injury respectively

[2]Weight and therefore BMI are self-reported values at the time of recruitment for both groups

VEGFA, Vascular endothelial growth factor A; BMI, body mass index; cm, centimetres; kg, kilograms; m, metres

[3]Post-hoc analysis: not significant $p>0.501$

[4]P-values adjusted for sex

[5]P-values adjusted for sex and age

Supplementary Table S3.4: *VEGFA* rs2010963 (-634 G>C) genotype associations on participant characteristics of the combined asymptomatic control (CON) and chronic Achilles Tendinopathy (TEN) groups of both cohorts.

	GG (n = 81)	**GC** (n = 82)	**CC** (n = 17)	**p-value**
Age (years) [1]	41.6 ± 12.1 (150)	39.1 ± 12.7 (156)	40.9 ± 13.0 (42)	0.204
Sex (% male)	68.1 (109)	59.1 (97)	59.1 (26)	0.198
Height (cm)	176.2 ± 9.1 (151)	174.4 ± 8.8 (148)	173.2 ± 9.9 (38)	0.104
Weight (kg) [2]	75.0 ± 16.5 (154)	73.5 ± 12.8 (152)	75.0 ± 16.1 (39)	0.648
BMI ($kg.m^{-2}$) [2]	24.1 ± 3.9 (151)	24.1 ± 3.4 (153)	24.5 ± 3.8 (40)	0.747

Except for sex, which is expressed as a percentage, values are expressed as mean ± standard deviation

P-values in bold typeset indicates significance ($p<0.05$)

[1]Age for the CON and TEN groups are at the time of recruitment and first injury respectively

[2]Weight and therefore BMI are self-reported values at the time of recruitment for both groups

VEGFA, Vascular endothelial growth factor A; BMI, body mass index; cm, centimetres; kg, kilograms; m, metres

Supplementary Table S3.5: *KDR* rs1870377 (1719 T>A) genotype associations on participant characteristics of the combined asymptomatic control (CON) and chronic Achilles Tendinopathy (TEN) groups of both cohorts.

	TT (n = 96)	**TA** (n = 65)	**AA** (n = 13)	**p-value**
Age (years) [1]	40.4 ± 12.6 (190)	40.0 ± 12.3 (125)	43.7 ± 12.1 (21)	0.450
Sex (% male)	61.5 (123)	63.9 (85)	69.6 (16)	0.724
Height (cm)	174.9 ± 9.0 (186)	174.6 ± 9.1 (120)	179.3 ± 9.4 (20)	0.098
Weight (kg) [2]	74.2 ± 16.2 (189)	73.8 ± 12.6 (125)	81.8 ± 16.0 (20)	0.080
BMI ($kg.m^{-2}$) [2]	24.4 ± 4.0 (186)	24.1. ± 3.1 (126)	25.1 ± 3.8 (21)	0.518

Except for sex, which is expressed as a percentage, values are expressed as mean ± standard deviation

P-values in bold typeset indicates significance

[1]Age for the CON and TEN groups are at the time of recruitment and first injury respectively

[2]Weight and therefore BMI are self-reported values at the time of recruitment for both groups

KDR, Kinase Domain Receptor, BMI, body mass index; cm, centimetres; kg, kilograms; m, metres

Supplementary Table S3.6: *KDR* rs2071559 (-604 G>A) genotype associations on participant characteristics of the combined asymptomatic control (CON) and chronic Achilles Tendinopathy (TEN) groups of both cohorts.

	GG (n = 51)	**GA** (n = 76)	**AA** (n = 54)	**p-value**
Age (years) [1]	41.6 ± 12.7 (107)	40.1 ± 11.9 (154)	39.3 ± 13.3 (89)	0.412
Sex (% male)	60.2 (68)	65.5 (108)	63.0 (58)	0.627
Height (cm)	174.0 ± 9.0 (100)	175.8 ± 9.3 (154)	175.1 ± 8.7 (85)	0.305
Weight (kg) [2]	73.6 ± 13.6 (103)	75.4 ± 16.6 (155)	73.2 ± 13.0 (89)	0.450
BMI ($kg.m^{-2}$) [2]	24.2 ± 3.6 (103)	24.1 ± 3.8 (156)	24.1 ± 3.3 (87)	0.990

Except for sex, which is expressed as a percentage, values are expressed as mean ± standard deviation

P-values in bold typeset indicates significance

[1]Age for the CON and TEN groups are at the time of recruitment and first injury respectively

[2]Weight and therefore BMI are self-reported values at the time of recruitment for both groups

KDR, Kinase Domain Receptor; BMI, body mass index; cm, centimetres; kg, kilograms; m, metres

Supplementary Table S3.7: Hardy-Weinberg Equilibrium (HWE) P-values for *VEGFA* rs699947 (-2578 C>A), and rs2010963, (-634 G>C), as well as *KDR* rs2071559 (-604 G>A) and *KDR* rs1870377 (1719 T>A) of the asymptomatic control (CON) and chronic Achilles Tendinopathy (TEN) groups of both cohorts, as well the combined CON and TEN participants.

	CON	**TEN**	**CON & TEN**
***VEGFA* rs699947**	0.236	0.450	0.833
***VEGFA* rs2010963**	0.434	0.613	0.818
***KDR* rs1870377**	1.000	0.695	0.888
***KDR* rs2071559**	0.556	**0.037**	**0.047**

P-values in bold typeset indicates significance

VEGFA, Vascular endothelial growth factor A; *KDR*, Kinase Domain Receptor

Supplementary Table S4.1: *VEGFA* rs699947 (-2578 C>A) genotype associations on the characteristics of participants with a history of chronic Achilles Tendinopathy (TEN).

	CC (n = 18)	**CA** (n = 37)	**AA** (n = 22)	**P-value**
Age (years) [1]	39.9 ± 14.3	40.1 ± 11.1	46.0 ± 10.2	0.135
Sex (% male)	50.0	51.4	77.3	0.105
Height (cm)	172.0 (168.0; 185.0)	173.5 (165.0; 177.6))	175.0 (171.0; 184.0)	0.063
Weight (kg) [2]	80.0 (67.5; 84.5)	70.0 (62.0; 80.0)	76.0 (69.5; 84.7)	0.133
BMI ($kg.m^{-2}$) [2]	24.0 (21.6; 28.3)	24.5 (21.7; 26.9)	24.3 (23.2; 25.9)	1.000

Except for sex, which is expressed as a percentage, values are expressed as average ± standard deviation or median (Q1; Q3) for parametric and non-parametric data respectively

The number of participants (n) in each genotype group is indicated

VEGFA, Vascular endothelial growth factor A; BMI, body mass index; cm, centimetres; kg, kilograms; m, metres

[1]Age is at the time of first injury

[2]Weight and therefore BMI are self-reported values at the time of recruitment

Supplementary Table S4.2: *VEGFA* rs2010963 (-634 G>C) genotype associations on the characteristics of participants with a history of chronic Achilles Tendinopathy (TEN).

	GG (n = 37)	**GC** (n = 32)	**CC** (n = 8)	**P-value**
Age (years) [1]	45.3 ± 10.2	37.1 ± 11.7	44.6 ± 14.5	**0.012** [3]
Sex (% male)	75.7	37.5	62.5	**0.006** [4]
Height (cm)	175.2 ± 7.0	172.0 ± 9.3	175.0 ± 9.6	0.273
Weight (kg) [2]	74.6 ± 12.9	73.4 ± 15.6	77.9 ± 9.4	0.716
BMI ($kg.m^{-2}$) [2]	24.4 ± 3.3	24.5 ± 4.4	25.5 ± 3.6	0.767

Except for sex, which is expressed as a percentage, values are expressed as average ± standard deviation

The number of participants (n) in each genotype group is indicated

P-values in bold typeset indicates significance ($p<0.05$)

VEGFA, Vascular endothelial growth factor A; BMI, body mass index; cm, centimetres; kg, kilograms; m, metres

[1]Age is at the time of first injury

[2]Weight and therefore BMI are self-reported values at the time of recruitment

[3]Post-hoc analysis: GG vs GC, **P = 0.004**; GC vs CC P =0.095 and GG vs CC P = 0.88

[4s]GG vs GC + CC (43.9% male)

Supplementary Table S4.3: *KDR* rs2071559 **(**-604 G>A) genotype associations on the characteristics of participants with a history of chronic Achilles Tendinopathy (TEN).

	GG (n = 22)	**GA** (n = 31)	**AA** (n = 24)	**P-value**
Age (years) [1]	42.5 (36.3; 53.0)	43.0 (33.8; 53.3)	41.0 (25.8; 47.8)	0.247
Sex (% male)	45.5	67.7	58.3	0.268
Height (cm)	171.2 ± 8.3	176.6 ± 8.5	172.5 ± 7.4	0.054
Weight (kg) [2]	72.1 ± 13.2	79.3 ± 14.5	70.5 ± 11.8	**0.041** [3]
BMI ($kg.m^{-2}$) [2]	24.6 (21.8; 27.3)	24.4 (23.0; 27.3)	23.8 (21.2; 24.8)	0.302

Except for sex, which is expressed as a percentage, values are expressed as average ± standard deviation or median (Q1; Q3) for parametric and non-parametric data respectively

The number of participants (n) in each genotype group is indicated

The number of participants (n) in each genotype group is indicated

P-values in bold typeset indicates significance ($p<0.05$)

KDR, Kinase Domain Receptor; BMI, body mass index; cm, centimetres; kg, kilograms; m, metres

[1]Age is at the time of first injury

[2]Weight and therefore BMI are self-reported values at the time of recruitment

[3]Post-hoc analysis: GA vs AA, **P = 0.018**; GA vs GG P =0.064 and GG vs AA P = 0.693

Supplementary Table S4.4: *KDR* rs1870377 (1719 T>A) genotype associations on the characteristics of participants with a history of chronic Achilles Tendinopathy (TEN).

	TT (n = 46)	**TA** (n = 23)	**AA** (n = 7)	**P-value**
Age (years) [1]	41.4 ± 11.3	43.7 ± 13.6	39.1 ± 9.9	0.608
Sex (% male)	56.5	65.2	57.1	0.636 [3]
Height (cm)	174.1 ± 9.0	172.8 ± 7.7	175.5 ± 7.2	0.744
Weight (kg) [2]	74.1 ± 14.2	73.1 ± 9.2	84.6 ± 20.6	0.173
BMI ($kg.m^{-2}$) [2]	24.5 ± 3.6	24.2 ± 3.3	27.3 ± 5.6	0.175

Except for sex, which is expressed as a percentage, values are expressed as average ± standard deviation

KDR, Kinase Domain Receptor; BMI, body mass index; cm, centimetres; kg, kilograms; m, metres

[1]Age is at the time of first injury

[2]Weight and therefore BMI are self-reported values at the time of recruitment for both groups

[3]TT vs TA+ AA (63.3% male)

Supplementary Table 4.5: *VEGFA* rs699947 (-2578 C>A), and rs2010963, (-634 G>C), as well as *KDR* rs2071559 **(**-604 G>A) and *KDR* rs1870377 (1719 T>A) genotype associations on the diameter of the dominant injured Achilles tendon in TEN participants with bilateral Achilles tendinopathy and the maximum (max) diameter of the two injured tendons.

				Dominant for Bilateral		**Max for Bilateral**	
SNP		**Genotype**	**n**	**Diameter** (mm)	**P-value**	**Diameter** (mm)	**P-value**
VEGFA	**rs699947**	**CC**	15	7.6 ± 2.7		7.6 ± 2.7	
		CA	21	7.2 ± 2.0	0.838	7.4 ± 2.1	0.939
		AA	17	7.2 ± 2.2		7.3 ± 2.2	
VEGFA	**rs2010963**	**GG**	25	6.8 (5.4; 8.2)		6.9 (5.5; 8.6)	
		GC	22	6.6 (5.2; 8.4)	0.528	6.8 (5.4; 8.4)	0.570
		CC	6	8.1 (6.5; 10.1)		8.1 (6.5; 10.1)	
KDR	**rs2071559**	**GG**	11	6.1 (5.3; 7.1) [a]	**0.001**	6.1 (5.4; 7.1) [a]	**<0.001**
		GA	23	8.1 (7.2; 9.6) [a,b]	[a] **0.027**	8.1 (7.2; 9.7) [a,b]	[a] **0.014**
		AA	19	5.4 (4.9; 7.4) [b]	[b] **0.002**	5.4 (4.9; 7.4) [b]	[b] **0.001**
KDR	**rs1870377**	**TT**	30	7.2 (6.2; 8.4)		7.2 (6.2; 8.7)	
		TA	17	6.0 (5.3; 8.9)	0.363	6.1 (5.4; 9.8)	0.498
		AA	5	7.4 (4.7; 8.1)		7.4 (4.7; 8.1)	

Values are expressed as average ± standard deviation or median (Q1; Q3) for parametric and non-parametric data respectively

SNP, single nucleotide polymorphism; *VEGFA*, Vascular endothelial growth factor A; *KDR*, Kinase Domain Receptor

P-values in bold typeset indicates significance

[a] GG vs GA and AA

[b] AA vs GA and GG

Supplementary Table 4.6: *VEGFA* rs699947 (-2578 C>A), *VEGFA* rs2010963, (-634 G>C), *KDR* rs2071559 **(**-604 G>A) and *KDR* rs1870377 (1719 T>A) genotype associations on the presence (Yes) and absence (No) of abnormal ultrasound findings in dominant tendon of TEN participants with bilateral Achilles tendinopathy and the tendon with the maximum (Max) abnormal ultrasound findings.

			Dominant for Bilateral			Max for Bilateral		
SNP		**Genotype**	**Yes**	**No**	**P-value**	**Yes**	**No**	**P-value**
VEGFA	**rs699947**	**CC**	22.6 (7)	26.5 (9)		23.5 (8)	25.8 (8)	
		CA	48.4 (15)	41.2 (14)	0.841	50.0 (17)	38.7 (12)	0.630
		AA	29.0 (9)	32.4 (11)		26.5 (9)	35.5 (11)	
VEGFA	**rs2010963**	**GG**	45.2 (14)	50.0 (17)		44.1 (15)	51.2 (16)	
		GC	41.9 (13)	44.1 (15)	0.805 [a]	44.1 (14)	41.9 (13)	0.803 [a]
		CC	12.9 (4)	5.9 (2)		11.8 (4)	6.5 (2)	
KDR	**rs2071559**	**GG**	25.8 (8)	29.4 (10)		26.5 (9)	29.0 (9)	
		GA	51.6 (16)	29.4 (10)	0.149	52.9 (18)	25.8 (8)	**0.048**
		AA	22.6 (7)	41.2 (14)		20.6 (7)	45.2 (14)	
KDR	**rs1870377**	**TT**	64.5 (20)	57.6 (19)		66.7 (22)	54.8 (17)	
		TA	25.8 (8)	33.3 (11)	0.615 [b]	24.2 (8)	35.5 (11)	0.443 [b]
		AA	9.7 (3)	9.1 (3)		9.1 (3)	9.7 (3)	

Values are expressed as a percentage with the number of participants (n) in parentheses

SNP, single nucleotide polymorphism; *VEGFA,* Vascular endothelial growth factor A; *KDR*, Kinase Domain Receptor

P-values in bold typeset indicates significance

[a] GG vs GC and CC

[b] TT vs TA and AA

Appendix A

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E53-46 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6626
Email: shuretta.thomas@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

29 September 2016

HREC REF: 279/2016

Prof M Collins
Human Biology
Sport Science Institute

Dear Prof Collins

PROJECT TITLE: AETIOLOGY OF PAIN IN CHRONIC ACHILLES TENDINOPATHY (PHD CANDIDATE - MS MKUMBUZI)

Thank you for submitting your response to the Faculty of Health Sciences Human Research Ethics Committee dated 12 September 2016.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30th September 2017.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval before the research may occur.

The HREC acknowledge that the student Sharon Nonhlanhla Mkombuzi will also be involved in this study.

Yours sincerely

Signed by candidate

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical

Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI),and Declaration of Helsinki (2013) guidelines.
The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938) This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30.11.2022
☐ Not approved	See attached comments		
Signature Chairperson of the HREC/ Designee	Signed by candidate	Date Signed	16/11/21

Note: Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za.
Please clarify your plan for research-related activities during COVID-19 lockdown.
Please use the latest form found on our website:
http://www.health.uct.ac.za/fhs/research/humanethics/forms

Comments to PI from the HREC

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	**11 November 2021**		
HREC REF Number	**279/2016**	Current Ethics Approval was granted until	**25 November 2021**
Protocol title	**AETIOLOGY OF PAIN IN CHRONIC ACHILLES TENDINOPATHY.**		
Protocol number (if applicable)			
Are there any sub-studies linked to this study?		X Yes	☐ No
If yes, could you please provide the HREC Reference number for all sub-studies? **Note:** A separate FHS016 must be submitted for each sub-study.	**311/2018**		
Principal Investigator	**Prof. Malcolm Collins**		

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Appendix B

Division of Physiological Sciences
Department of Human Biology

Health, Physical Activity, Lifestyle, Sport (HPALS) Research Centre

Faculty of Health Sciences
University of Cape Town
Private Bag X3 Observatory
7935
South Africa

WHY DO SOME ATHLETES EXPERIENCE THE PAIN OF ACHILLES TENDINOPATHY WHILE OTHERS WITH THE SAME INJURY DO NOT?

PARTICIPANTS WANTED FOR UCT RESEARCH

A comparison of the genetic differences between athletes with painful chronic Achilles tendinopathy and those who are pain free

The aim of this study is to determine the association between variations in certain genes and pain in chronic Achilles tendinopathy.

You can volunteer for this study if you:-

- are at least 18 years old,
- with a body mass index of less than 30 kg/m^2,
- do not have a history of Achilles tendon rupture,
- have not had surgery to the legs in the past two years,
- do not take any chronic medication that can affect tendons,
- Have pain in the Achilles tendon area for at least 6 months **OR** have no history of Achilles tendon injury

What is involved?

- Complete questionnaires and pain rating scales
- Donate a small blood sample (5ml=1 teaspoon)
- Have ultrasound scans taken of your Achilles tendons
- Have experimental cold and pressure pain induced

What are the benefits?

- Receive personalised feedback of your Achilles tendon health

If you are interested in taking part in the study and would like additional information, please contact:
Christina Brazier at brzchr002@myuct.ac.za or on 071 279 8817

Appendix C

Division of Physiological Sciences
Department of Human Biology

Health, Physical Activity, Lifestyle, Sport (HPALS) Research Centre

Faculty of Health Sciences
University of Cape Town
Private Bag X3 Observatory
7935
South Africa

THE AETIOLOGY OF PAIN IN CHRONIC ACHILLES TENDINOPATHY

PARTICIPANT INFORMATION SHEET AND INFORMED CONSENT FORM

Dear Volunteer,

Thank you for agreeing to participate in this study to be conducted by researchers from the Health through Physical Activity, Lifestyle and Sport Research Centre (HPALS) within the Department of Human Biology at the University of Cape Town.

Why are we doing this study?

Achilles Tendinopathy (ATP) - pain and dysfunction in the Achilles tendon - is a common presentation in sport. The occurrence of overuse ATP in sport has risen enormously due to the increase in participation in sporting endeavours by recreational athletes and the increased duration and intensity of training regimens for professional athletes. This injury may be painless without symptoms in some individuals but often pain is the major complaint and the reason for seeking healthcare in most. However, the precise mechanisms for this pain are poorly understood. As such, most current treatment options for pain in tendinopathy are not very effective.

Some researchers have suggested that our genes may affect, at least in part, the human response to musculoskeletal pain**.** Genes are the basic units of inheritance. They are passed on from our parents and carry information on various personal characteristics such as hair colour, personality and disease risk. In an attempt to determine whether there is a genetic basis for the pain experienced in ATP and hence explain why some athletes experience it more than others, we are interested in studying whether certain genes are associated with pain in chronic tendinopathies. Todo so, we will compare various features of tendons from athletes with painful tendinopathy against those with no pain.

Achilles tendinopathy, a chronic painful injury is debilitating and is challenging for clinicians and athletes to manage and we are interested in the question ‘What are the differences between athletes who experience the pain ofAchilles tendinopathy and those with the same injury who do not?’

What are the aims of this study?

The aims of this study are:

1. To determine the association between pain and structural changes in painful and pain free Achilles tendons using ultrasound scans.

2. To investigate the association between genetic variations in selected genes and pain in chronic Achilles tendinopathy.
3. To investigate the association between variations in selected genes and experimentally induced pain thresholds.

We anticipate that the information generated from this study will reveal which athletes are at risk of experiencing chronic Achilles tendon pain thus providing some knowledge on the possible mechanisms of pain in tendinopathy and thus allowing development of interventions to manage pain in athletes with chronic Achilles tendinopathy.

Who can take part in this study?

- If you are a physically active individual with Achilles tendon pain of at least 6 months' duration.

or

- If you are a physically active individual without Achilles tendon pain.

To volunteer you need to be above 18 years old.

If you (i) have used fluroquinolone antibiotics or corticosteroid injections to the Achilles tendon in the past 12 months (ii) have a history of Achilles tendon rupture, (iii) are taking any chronic medications (iv) have a body massindex $\geq$30kg m^{-2} plus a waist circumference $\geq$102cm (v) have had surgery to the lower limbs in the last two years and (vi) have lower limb deformities, you shall not be eligible for this study.

What will happen if you do decide to take part in this study?

The researcher will explain the study in detail to you, including the risks and benefits associated with participating. You will be free to ask any questions you may have relating to the study. Should you agree to participate, you will sign a consent form and be asked to complete questionnaires detailing personal information, your medical history and training. These will include questionnaires on your current pain on recruitment and the effect the pain has on your training schedule. This should take about 30 minutes to complete. On completion of these forms, a small blood sample (5ml=1 teaspoon) will be drawn from your forearm and stored in a tube until further analysis. Afterwards, the radiologist will then take ultrasound scans of your Achilles tendons, a process that should take about 15 minutes.

We also want to measure your pain thresholds to experimental pain. To do this, the investigator will ask you to immerse the hand opposite the painful tendon in ice water until the cold becomes painful. You then be asked to rate this pain on a pain scale. Following which, point pressure will then be applied to your painful Achilles tendon using a pressure probe, when this pressure becomes painful you will signal the researcher and they will stop. This should take no more than 10 minutes of your time.

What are the risks and discomforts of this study?

The potential risks associated with blood collection technique from the forearm veins are: infection, delayed healing, haematoma, physical pain, mental discomfort and injury to a nerve or a vessel. These risks are small and will be minimized by the use of trained phlebotomists, use of sterile techniques and the use of disposable, single usematerials. The risks associated with the experimental pain procedure are mild discomfort from the cold water in the ice bath and from the pressure probe. However, as soon as either of these becomes uncomfortable for you, the researcher will immediately stop the protocol. The completion of questionnaires is not associated with any risk. Questionnaire and other clinical data (paper and electronic) will be kept confidential and secure, and will not be made available to any party other than the research team without your consent.

What are the benefits of taking part in this study?

There is no direct benefit to you for taking part in this study. You will however receive the results of the ultrasound scans of your Achilles tendons that you may use to inform future clinical decisions by your medical practitioner. You will also receive general feedback at the end of the study describing the genetic basis of pain in Achilles tendinopathy.

What are the ethical considerations?

The UCT Human Research Ethics Committee (contact information below) has approved this study. This study will be performed in accordance with the principles of the Declaration of Helsinki (2013, Fortaleza, Brazil), International Conference on Harmonisation (ICH) and South African Good Clinical Practice (GCP) guidelines and the laws of South Africa. The study will be covered by the no-fault insurance policy of the University of Cape Town. You will not be included in the study unless you have signed a consent form, after the investigator has provided substantial verbal and written explanation of the study, including risk factors. You will be informed that your participation in the study is entirely voluntary and that you have the right to withdraw from the study at any time without stating a reason. The investigator may also withdraw you from the study at any time. All the information collected during the trial will be stored in a computer database in a secure facility, will be kept confidential and will only be used for scientific purposes. Your anonymity will be ensured should the data be published**. At the end of the study on genetic risk factors for chronic Achilles tendinopathy, any samples takenfrom you will be destroyed.**

What if something goes wrong?

The University of Cape Town has insurance cover for the event that research-related injury or harm results from your participation in the trial. The insurer will pay all reasonable medical expenses in accordance with the South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI) in the event of an injury or side effect resulting directly from your participation in the trial. You will not be required to prove fault on the part of the University.

The University **will not be liable** for any loss, injuries and/or harm that you may sustain where the loss is caused by:

- The use of unauthorised medicine or substances during the study
- Any injury that results from you not following the protocol requirements or the instructions that the study doctor may give you
- Any injury that arises from inadequate action or lack of action to deal adequately with a side effect or reaction to the study medication
- An injury that results from negligence on your part

By agreeing to participate in this study, you do not give up your right to claim compensation for injury where you can prove negligence, in separate litigation. In particular, your right to pursue such a claim in a South African court in terms of South African law must be ensured. Note, however, that you will usually be requested to accept that payment made by the University under the SA GCP guideline 4.11 is in full settlement of the claim relating to the medical expenses.

An injury is considered trial-related if, and to the extent that, it is caused by study activities. You must notify the study doctor immediately of any side effects and/or injuries during the trial, whether they are research- related or other related complications.

UCT reserves the right not to provide compensation if, and to the extent that, your injury came about because you chose not to follow the instructions that you were given while you were taking part in the study. Your right in law to claim compensation for injury where you prove negligence is not affected. Copies of these guidelines are available on request.

Who do I speak to (or contact) if I have any questions about the study?

Should you have any ethical concerns or questions about the study, please contact the Human Research Ethics Committee:

Faculty of Health Sciences - Research Ethics Committee Room E52-
24, Old Main Building, Groote Schuur Hospital Observatory, 7925
Tel: (021) 406 6338
Fax: (021) 406 6441
Email: nosi.tywabi@uct.ac.za

Should you have any queries directly related to the study itself, please contact any of the investigators:

Prof. Malcolm Collins, PhD	Prof Alison September, PhD	Ms. Christina Brazier
(021) 650 4574	(021) 650 4559	brzchr002@myuct.ac.za
malcolm.collins@uct.ac.za	alison.september@uct.ac.za	

CONSENT

I, the undersigned, have been fully informed about the University of Cape Town's study entitled **"Aetiology of pain in chronic Achilles tendinopathy"** to be conducted by researchers from the Health through Physical Activity, Lifestyle and Sport Research Centre (HPALS) within the Department of Human Biology.

- I agree to donate five millilitres of venous blood which will be used for the extraction and analysis of genetic material (DNA). I agree that this blood sample will be drawn by a nurse, physician or trained phlebotomist.
- I agree to complete personal particulars, sporting participation, personal and family medical history, as well as, pain rating questionnaires.
- I agree to have cold and pressure pain administered to my hand and ankle respectively
- I agree to have an ultrasound scan(s) of my Achilles tendon(s) taken.

I have been fully informed about the risks inherent in participation in this trial. I have had the opportunity to ask questions about the study and had them answered to my satisfaction. I understand that all the information collected during the study will be treated confidentially, will only be used for scientific research purposes and that my name and personal particulars will not be released under any circumstances.

I have been informed that I will be free to withdraw from the study at any time if I so wish without explanation. I will be free to ask any questions about the procedures and results of the study. I understand that I will receive, where applicable, feedback pertaining to the general results of the study once the entire study has been completed.

I agree to participate in the study.

Participant:

Full name	Signature	Date

Investigator:

Full name	Signature	Date

Witness:

Full name	Signature	Date

Appendix D

Division of Physiological Sciences
Department of Human Biology

Health, Physical Activity, Lifestyle, Sport (HPALS) Research Centre

Faculty of Health Sciences
University of Cape Town
Private Bag X3 Observatory
7935
South Africa

PARTICIPANT QUESTIONNAIRES

Instructions

Please answer each question by filling in the details in the allocated space or checking one or more of the option boxes.

Please complete all sections A to H

Section A	Personal, demographic, and occupational details	Pages 2-3
Section B	Current and past sporting details	Pages 4-5
Section C	Lifestyle and habits history	Page 6
Section D	General personal medical history	Pages 7-8
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Section F	History of medication use	Page 10
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Section A: Personal details (to be completed and kept separately from the rest of the questionnaire)		
Surname		
First Name		
Postal Address		Postal/ Zip Code
E-mail address		
Cell phone or daytime phone number		

Participant code:……

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Section A: Demographic and occupational details			
Date of birth	(YYYY-MM-DD)		
Age	years	Sex	☐ Male ☐ Female
Height	cm	Weight	kg
Ethnic group (Only Required and Used for Research Purposes)	☐ Black/African ☐ White ☐ Indian ☐ Mixed Ancestry (Coloured) ☐ Asian ☐ Other		
Ancestry: Tribal or national background (eg Xhosa, Dutch, Zulu, German, Italian)	Father: ☐ Unknown Mother: ☐ Unknown		
Country of Birth			
Dominant Hand	☐ Left ☐ Right ☐ Both	Dominant Leg	☐ Left ☐ Right ☐ Both
Current Occupation			
What **percentage** of your **working** day is spent in the following activities?	Sitting: _____ % Standing: _% Walking (Lower body activity) _% Manual Labour (upper and lower body activity) _%		
Occupation prior to development of tendinopathy?		☐ N/A	
Prior to tendinopathy, did your occupation involve lower or upper limb activity?		☐ Yes ☐ No ☐ N/A	
If yes please indicate which arms/legs.	☐ Right arm ☐ Left arm ☐ Both legs ☐ N/A ☐ Right leg ☐ Left leg ☐ Both arms ☐ N/A		

Participant code:……

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Section B: Current and past sporting details Please record your current and past sporting activities in order of importance Use an additional form if you participate(d) in more than 6 sports			
	Main sport 1	**Other sport 2**	**Other sport 3**
Type of current and previous sport(s) you have participated in (please name)			
Current or past participation	☐ Current ☐ Past	☐ Current ☐ Past	☐ Current ☐ Past
Year started participation			
Number of years involved in the sport			
Years in competitive sport			
Highest level competed at	☐ Professional ☐ Amateur	☐ Professional Amateur ☐	☐ Professional Amateur ☐
Hours of training per week during:- ▪ last 0-3 months			
▪ last 4-12 months			
▪ last 13-24 months			

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	Other sport 4	**Other sport 5**	**Other sport 6**
Type of current and previous sport(s) you have participated in (please name)			
Current or past participation	☐ Current ☐ Past	☐ Current ☐ Past	☐ Current ☐ Past
Year started participation			
Number of years involved in the sport			
Years in competitive sport			
Highest level competed at	☐ Professional ☐ Amateur	☐ Professional Amateur ☐	☐ Professional Amateur ☐
Hours of training per week during:- ▪ last 0-3 months			
▪ last 4-12 months			
▪ last 13-24 months			

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Section C: Lifestyle and habits history	
Please indicate your smoking status	☐ Current smoker ☐ Ex-smoker ☐ Never smoked
If you answered yes, (past or current smoker) please answer the questions on the right on the right	How many years did or have you smoked?: If stopped, how many years ago: What is (was) the average number of cigarettes per day:
On average, how much alcohol do you drink per week (tots, glasses) of spirits, wine or beer?	_____glasses beer/cider per week _____ glasses wine per week _____ tots of spirits per week

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Section D: General Personal Medical History		
Do you currently suffer from any of these medical conditions: (if **none** then select "None of the above")		
☐ High blood pressure	☐ Angina/Heart attack	☐ Asthma
☐ Emphysema	☐ Rheumatoid arthritis Elevated	☐ Osteoarthritis (wear & tear)
☐ Malignant disease(cancer) If **Yes**, what type? _____	☐ blood cholesterol Diabetes mellitus	☐ Adrenal disorders
	☐ Renal disease	☐ Thyroid disorders
	☐ **None of the above**	☐ Amyloidosis
Do you currently suffer from any other Connective Tissue, Rheumatological Or Muscle Diseases & Disorders?	☐ Yes ☐ No	If **Yes**, please select from the list below:
List of some Connective Tissue and/or Rheumatic Diseases and Disorders		
☐ Ankylosing spondylitis	☐ Marfan syndrome	☐ Reiter's syndrome
☐ Aspartylglycosaminuria (AGU)	☐ Menkes kinky hair syndrome	☐ Relapsing polychrondritis
☐ Bechet's syndrome	☐ Mucopolysaccharidoses	☐ Rhabdomyolysis
☐ Crohn's disease	☐ Myopathies and dystrophies	☐ Scleroderma
☐ Discoid lupus erythematosus	☐ Ochronosis (homocystinuria)	☐ Sjogren's syndrome
☐ Ehlers-Danlos syndrome (EDS)	☐ Osteogenesis imperfecta (OI)	☐ Systemic lupus erythematosus
☐ Eosinophilic fasciites	☐ Polyarteritis nodosa Polymyalgia	☐ Systemic sclerosis
☐ Giant cell (temporal) arthritis	☐ rheumatica Polymyositis &	☐ Wegener's granulomatosis Other -
☐ Gout	☐ dermatomyositis Pseudogout	☐ If **Yes**, what type? _____
☐ Hypersentive vasulatis	☐ Reactive arthritis	
☐ Lipid storage diseases		

What surgical operations have you had? (please list and give dates)	**Operation**	**Date (year)**
Example:		
Appendix removed 1993		
ACL reconstruction Dec 2005		

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Do you suffer/have you suffered from (a) chronic pain condition(s)?	☐ Yes ☐ No	If **Yes,** please select from the list below:

☐ Low back pain	☐ Ishaemic muscular pain	☐ Vulvar pain
☐ Chronic pelvic pain	☐ Trigeminal neuralgia	☐ Herniated Intervertebral disc
☐ Headaches / migraines	☐ Fibromylagia	☐ Pancreatitis
☐ Phantom limb pain	☐ Congenital insensitivity to pain	☐ Paroxysmal extreme pain disorder
☐ Chronic widespread pain	☐ Erythromelalgia	☐ Altered response to pain medication
☐ Peripheral neuropathy	☐ Chronic inflammation	☐ Other - If **Yes**, what type?
☐ Increased pain sensitivity	☐ Lumbago-sciatica	_____

Menstrual history: (females only)		
At what age did you start menstruating? (years)		
Are you currently using any type of contraception?	☐ Yes ☐ No	
If Yes, what type of contraception are you using?	☐ Pill ☐ Injection ☐ IUD ☐ Rods ☐ Other (Specify)	
Have you ever experienced labour/childbirth?	☐ Yes ☐ No	
Do you suffer/have you suffered from any painful menstrual/ hormonal conditions	☐ Yes ☐ No	If **Yes**, please select from the list below

☐ Dysmenorrhoea (period pain)	☐ Endometriosis
☐ Amenorrhoea (no period)	☐ Polycystic Ovarian Syndrome (PCOS) Other -
☐ Menorrhagia (heavy bleading)	☐ If **Yes**, please specify?
	_____

Are you currently?	
	☐ Pre-menopausal (±12 cycles/year at intervals of 23-33 days & bleeding lasts 3-7 days)
	☐ Menopausal (cycles are irregular and less frequent)
	☐ Post-menopausal (n o longer menstruating)

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Section E: Family Medical History

Have any of your blood (biological) relatives ever had the following?

Please tick yes or no. If yes, please tick the relationship of that person to you (You may tick more than one of the relationship blocks).

Description		If **Yes**, please indicate the relationship			
Chronic **Achilles** tendon injury	☐ Yes ☐ No	☐ Father ☐ Sister ☐ Child	☐ Mother ☐ Brother	☐ Uncle/Aunt ☐ Cousin	☐ Grandfather ☐ Grandmother ☐ Grandchild
Achilles tendon rupture	☐ Yes ☐ No	☐ Father ☐ Sister ☐ Child	☐ Mother ☐ Brother	☐ Uncle/Aunt ☐ Cousin	☐ Grandfather ☐ Grandmother ☐ Grandchild
Any other (not Achilles) tendon injury/rupture	☐ Yes ☐ No	☐ Father ☐ Sister ☐ Child	☐ Mother ☐ Brother	☐ Uncle/Aunt ☐ Cousin	☐ Grandfather ☐ Grandmother ☐ Grandchild
Anterior cruciate ligament (ACL) rupture	☐ Yes ☐ No	☐ Father ☐ Sister ☐ Child	☐ Mother ☐ Brother	☐ Uncle/Aunt ☐ Cousin	☐ Grandfather ☐ Grandmother ☐ Grandchild
Any other (NOT ACL) ligament injury/rupture	☐ Yes ☐ No	☐ Father ☐ Sister ☐ Child	☐ Mother ☐ Brother	☐ Uncle/Aunt ☐ Cousin	☐ Grandfather ☐ Grandmother ☐ Grandchild
Exercise associated muscle cramps	☐ Yes ☐ No	☐ Father ☐ Sister ☐ Child	☐ Mother ☐ Brother	☐ Uncle/Aunt ☐ Cousin	☐ Grandfather ☐ Grandmother ☐ Grandchild
Night muscle cramps	☐ Yes ☐ No	☐ Father ☐ Sister ☐ Child	☐ Mother ☐ Brother	☐ Uncle/Aunt ☐ Cousin	☐ Grandfather ☐ Grandmother ☐ Grandchild
Elevated blood cholesterol	☐ Yes ☐ No	☐ Father ☐ Sister ☐ Child	☐ Mother ☐ Brother	☐ Uncle/Aunt ☐ Cousin	☐ Grandfather ☐ Grandmother ☐ Grandchild
Arthritis	☐ Yes ☐ No	☐ Father ☐ Sister ☐ Child	☐ Mother ☐ Brother	☐ Uncle/Aunt ☐ Cousin	☐ Grandfather ☐ Grandmother ☐ Grandchild
Heart Disease	☐ Yes ☐ No	☐ Father ☐ Sister ☐ Child	☐ Mother ☐ Brother	☐ Uncle/Aunt ☐ Cousin	☐ Grandfather ☐ Grandmother ☐ Grandchild
Diabetes	☐ Yes ☐ No	☐ Father ☐ Sister ☐ Child	☐ Mother ☐ Brother	☐ Uncle/Aunt ☐ Cousin	☐ Grandfather ☐ Grandmother ☐ Grandchild

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Section F: History of Medication Use			
What medication, if any (including painkillers), are you currently using? (please list)	Name of medication		Days/Months/Years taken
Have you ever used oral corticosteroids (cortisone tablets)? (If **yes**, how long ago?)	☐ Yes ☐ No	☐ 0 to 3 months ago ☐ 4 to 6 months ago ☐ 7 to 12 months ago	☐ 13 to 24 months ago ☐ > 2 years ago
Have you ever been given an injection with corticosteroids? (If **yes**, how long ago?)	☐ Yes ☐ No	☐ 0 to 3 months ago ☐ 4 to 6 months ago ☐ 7 to 12 months ago	☐ 13 to 24 months ago ☐ > 2 years ago
Have you ever used fluoroquinoline antibiotics? (refer to the following list)	☐ Yes ☐ No	☐ 0 to 3 months ago ☐ 4 to 6 months ago ☐ 7 to 12 months ago	☐ 13 to 24 months ago ☐ > 2 years ago

List of some fluoroquinoline antibiotics (may be used in treatment of chlamydia, pneumonia, acute bronchitis, urinary tract infections, skin and soft tissue infection):

ADCO-CIPRIN	CIPROGEN	SANDOZ CIPROFLOXACIN
AVELON	CPL ALLIANCE CIPROFLOXACIN	TAFLOC
BACTIDRON	DYNAFLOC	TARIVID
CIFLOC	FACTIVE	TAVANIC
CIFRAN	FLOXIN	TEQUIN
CIPLA-CIPROFLOXACIN	MAXAQUIN	UNIQUIN
CIPLOXX	NOROXIN	UTIN-400
CIPRO-HEXAL	ORPIC	ZANOCIN
CIPROBAY		

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Section G: Past history of tendon, ligament and/or joint capsule injury

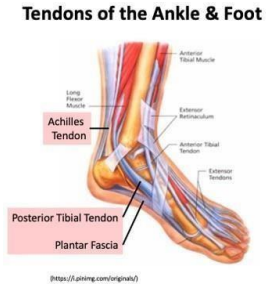
Please complete this section for each injury. If you have had more than one past injury additional forms will be available.

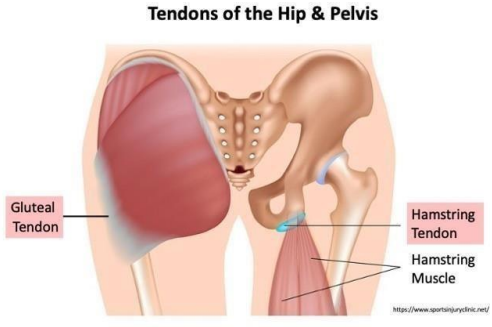
Have you **ever** in your suffered from **a tendon, ligament and/or joint capsule injury** (pain,swelling, stiffness, dislocation, instability) in any tendon (including Achilles tendon, knee tendons, and shoulder tendons), ligaments (partial or complete tear), or joint?	☐ Yes ☐ No

If **YES,** please complete the rest of the section below:-

If **NO**, continue completing the questionnaire from the next section

			Longstanding Pain (tendinopathy)		Acute Rupture	
	Tendon		**Left**	**Right**	**Left**	**Right**
Please tick which **tendon/s** you have injured? Also indicate the **number** of past longsatnding pain (tendinopathy) or acute tear/rupture you have had	Foot and ankle:	☐ Achilles tendon ☐ Tibialis posterior ☐ Plantar fascia				
	Knee:	☐ Patellar tendon ☐ Quadriceps tendon ☐ Hamstring tendon				
	Hip and Pelvis	☐ Hamstring tendon ☐ Gluteal tendon				
	Elbow and wrist:	☐ Wrist extensor tendon				
	Shoulder:	☐ Rotator cuff tendons				
	Other Tendons:	_____				





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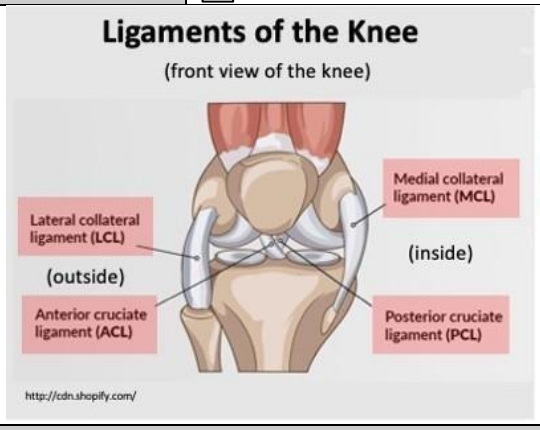
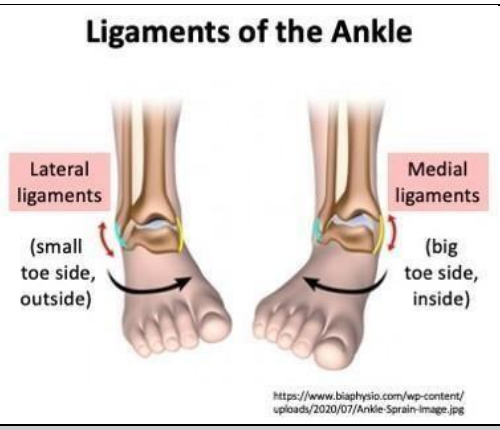
	Ligament	Sprain Left	Sprain Right	Tears Left	Tears Right
Please tick which **ligament/s** you have injured? (next column on the tright) Also indicate the **number** of past sprains or tears you have had	☐ Shoulder ligaments ☐ Elbow ligaments ☐ Wrist ligaments ☐ Finger ligaments ☐ Knee (ACL) ☐ Knee (MCL) ☐ Knee (PCL) ☐ Knee (LCL) ☐ Ankle lateral ligaments ☐ Ankle medial ligaments ☐ Spinal ligaments ☐ Other: _____				





Acute shoulder dislocation - The ball of the shoulder joint pops out of the cup-shaped socket that's part of your shoulder blade

Chronic shoulder instability - usually occurs when the lining of the shoulder joint (capsule), ligaments or labrum become stretched, torn or detached, allowing the ball of the shoulder joint to move either completely or partially out of the socket)

Have you ever suffered from any of the following joint capsule injuries?	Acute shoulder dislocation Chronic shoulder instability Chronic ankle instability Other: _____

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Section H: Medical details of tendon injuries				
How many times have you had tendon injuries? [1]Sudden onset is within a few seconds or minutes [2]Gradual onset is over days or weeks	Tendon Injured	Date of Injury	Acute or Chronic Injury	Sudden[1] or Gradual[2] Onset
	1			
	2			
	3			
	4			
	5			

Please complete this form for each tendon Injury you have had	
Injury Number (1,2,3,4,or 5)	
Which tendon did you injure?	☐ Rotator cuff tendon ☐ Supraspinatus ☐ Infraspinatus ☐ Teres minor ☐ Wrist extensor tendons ☐ Other (Specify _____) ☐ Gluteal tendon Hamstring ☐ tendon (hip) Patellar ☐ tendon ☐ Quadriceps tendon (knee) ☐ Hamstring tendon Achilles ☐ tendon ☐ Tibialis posterior ☐ Plantar fascia
Which side was injured?	☐ Left ☐ Right ☐ Both
Which region of your tendon was injured?	☐ Upper 1/3 ☐ Middle 1/3 ☐ Lower 1/3
To what extent was your Tendon ruptured?	☐ Complete ☐ Partial ☐ None
How were you injured? (e.g. sport, walking)	
Grade of injury **at the time** of injury	☐ pain only after exercise ☐ pain during exercise, but did not cause you to alter training ☐ pain during exercise, which causes you to alter training pain ☐ which causes you to stop training ☐ no pain ☐ not sure ☐ Other (Specify_____)

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Grade of injury **currently**	☐ pain only after exercise ☐ pain during exercise but did not cause you to alter training. pain ☐ during exercise, which causes you to alter training pain which ☐ causes you to stop training ☐ no pain not ☐ sure ☐ Other (Specify_____)
Which of the following symptoms were present **before** the injury	☐ Pain (less than 1 week) ☐ Stiffness ☐ Pain (1-4 weeks) ☐ Swelling ☐ Pain (> 4 weeks) ☐ None
Which of the following symptoms were present **after** the injury	☐ Pain (less than 1 week) ☐ Stiffness ☐ Pain (1-4 weeks) ☐ Swelling ☐ Pain (> 4 weeks) ☐ None
Which type of Tendon Disease were you diagnosed with e.g. Rupture, Tendinitis, etc.	
Diagnosed by	☐ Doctor Name:_____ ☐ Physiotherapist Name: Biokineticist ☐ Name:_____ ☐ Podiatrist Name: _____ ☐ Other Name: _____
How was it treated?	☐ Surgically ☐ Non- surgically

If non-surgically treatment please select from the list which modality/modalities alleviated the pain

☐ oral NSAIDs (such as ibuprofen, diclofenac, indomethacin) oral
☐ corticosteroids
☐ injected NSAIDs (such as diclofenac) plasma
☐ rich platelet (PRP) injections sclerosants (such
☐ as polidoconal) eccentric exercise
☐ electrotherapies (such as ultrasound, TENS, ShortWave Diathermy) heat
☐ therapy
☐ ice
☐ extracoporeal shockwave therapy (ESWT) orthotics
☐ acupunture
☐ rest
☐ taping/strapping massage
☐ activity modification
☐ Other (Specify_____)

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The VISA-A questionnaire: An index of the severity of Achilles tendinopathy

IN THIS QUESTIONNAIRE, THE TERM PAIN REFERS SPECIFICALLY TO PAIN IN THE ACHILLES TENDON REGION	
1. For how many minutes do you have stiffness in the Achilles region on first getting up?	**Points**
100 min ☐☐☐☐☐☐☐☐☐☐☐ 0 min 0 1 2 3 4 5 6 7 8 9 10	
2. Once you are warmed up for the day, do you have pain when stretching the Achilles tendon fully over the edge of a step? (Keeping knee straight)	
strong severe pain ☐☐☐☐☐☐☐☐☐☐☐ no pain 0 1 2 3 4 5 6 7 8 9 10	
3. After walking on flat ground for 30 minutes, do you have pain within the next 2 hours? (If unable to walk on flat ground for 30 minutes because of pain, score 0 for this question).	
strong severe pain ☐☐☐☐☐☐☐☐☐☐☐ no pain 0 1 2 3 4 5 6 7 8 9 10	
4. Do you have pain walking downstairs with a normal gait cycle?	
strong severe pain ☐☐☐☐☐☐☐☐☐☐☐ no pain 0 1 2 3 4 5 6 7 8 9 10	
5. Do you have pain during or immediately after doing 10 (single leg) heel raises from a flat surface?	
strong severe pain ☐☐☐☐☐☐☐☐☐☐☐ no pain 0 1 2 3 4 5 6 7 8 9 10	
6. How many single leg hops can you do without pain?	
No hops (0) ☐☐☐☐☐☐☐☐☐☐☐ 10 hops 0 1 2 3 4 5 6 7 8 9 10	
7. Are you currently undertaking sport or other physical activity? Not at ☐ all (0 points) ☐ Modified training ± modified competition (4 points) ☐ Full training ± competition but not at same level as when symptoms began (7 points) ☐ Competing at the same or higher level as when symptoms began (10 points)	

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8. Please complete **EITHER A, B or C** in this question
   - If you have no pain while undertaking Achilles tendon loading sports, please complete **Q8A** only.
   - If you have pain while undertaking Achilles tendon loading sports but it does not stop you from completing the activity, please complete **Q8B** only.
   - If you have pain that stops you from completing Achilles tendon loading sports, please complete **Q8C** only.

	Points
8A. If you have no pain while undertaking Achilles tendon loading sports, for how long can you train/practice? ☐ Nil (0 points) ☐ 1 to 10 mins (7 points) ☐ 11 to 20 min (14 points) ☐ 21 to 30 min (21 points) ☐ >30 min (30 points)	
OR 8B. If you have some pain while undertaking Achilles tendon loading sport, but it does not stop you from completing your training/practice for how long can you train/practice? ☐ Nil (0 points) ☐ 1 to 10 mins (4 points) ☐ 11 to 20 min (10 points) ☐ 21 to 30 min (14 points) ☐ >30 min (20 points)	
OR 8C. If you have pain that stops you from completing your training/practice in Achilles tendon loading sport, for how long can you train/practice? ☐ Nil (0 points) ☐ 1 to 10 mins (2 points) ☐ 11 to 20 min (5 points) ☐ 21 to 30 min (7 points) ☐ >30 min (10 points)	
Total Score_____/ 100	

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Short form McGill Questionnaire (sfMGQ)

Please rate your pain by marking the box besides the word that best describes your pain and its severity at present.

	None	Mild	Moderate	Severe
Throbbing	☐	☐	☐	☐
Shooting	☐	☐	☐	☐
Stabbing	☐	☐	☐	☐
Sharp	☐	☐	☐	☐
Cramping	☐	☐	☐	☐
Gnawing	☐	☐	☐	☐
Hot-burning	☐	☐	☐	☐
Aching	☐	☐	☐	☐
Heavy	☐	☐	☐	☐
Tender	☐	☐	☐	☐
Splitting	☐	☐	☐	☐
Tiring-Exhausting	☐	☐	☐	☐
Sickening	☐	☐	☐	☐
Fearful	☐	☐	☐	☐
Punishing-Cruel	☐	☐	☐	☐

Present pain intensity (please select)

☐ 0 no pain
☐ 1 mild
☐ 2 discomforting
☐ 3 distressing
☐ 4 horrible
☐ 5 excruciating

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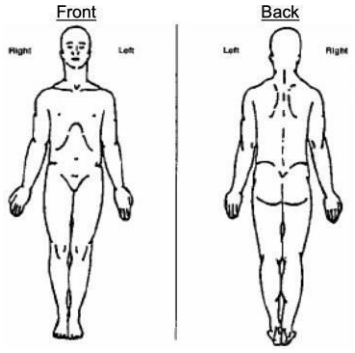
Brief Pain Inventory (Short Form)

1. Throughout our lives, most of us have had pain from time to time (such as minor headaches, sprains, and toothaches). Have you had pain other than those everyday kinds of pain today?

☐ Yes ☐ No

2. On the diagram, shade in the areas where you feel pain. Put an X on the area that hurts the most.



3. Please rate your pain by marking the box beside the number that best describes your pain at its **WORST** in the last 24 hours.

No Pain ☐☐☐☐☐☐☐☐☐☐☐ Pain as Bad as You Can Imagine

0 1 2 3 4 5 6 7 8 9 10

4. Please rate your pain by marking the box beside the number that best describes your pain at its **LEAST** in the last 24 hours.

No Pain ☐☐☐☐☐☐☐☐☐☐☐ Pain as Bad as You Can Imagine

0 1 2 3 4 5 6 7 8 9 10

5. Please rate your pain by marking the box beside the number that best describes your pain on the **AVERAGE.**

No Pain ☐☐☐☐☐☐☐☐☐☐☐ Pain as Bad as You Can Imagine

0 1 2 3 4 5 6 7 8 9 10

6. 6. Please rate your pain by marking the box beside the number that tells how much pain you have **RIGHT NOW.**

No Pain ☐☐☐☐☐☐☐☐☐☐☐ Pain as Bad as You Can Imagine

0 1 2 3 4 5 6 7 8 9 10

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7. What treatments or medications are you receiving for your pain?
8. In the last 24 hours, how much relief have pain treatments or medications provided? Please mark the box below the percentage that most shows how much **RELIEF** you have received.

No Relief ☐ 0% ☐ 10% ☐ 20% ☐ 30% ☐ 40% ☐ 50% ☐ 60% ☐ 70% ☐ 80% ☐ 90% ☐ 100% Complete Relief

9. Mark the box beside the number that describes how, during the past 24 hours, pain has interfered with your:

A: General Activity

Does Not Interfere ☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 Completely Interferes

B: Mood

Does Not Interfere ☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 Completely Interferes

C: Walking Ability

Does Not Interfere ☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 Completely Interferes

D: Normal Work (Includes Work both Outside and Inside the Home)

Does Not Interfere ☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 Completely Interferes

E: Relations with Other People

Does Not Interfere ☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 Completely Interferes

F: Sleep

Does Not Interfere ☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 Completely Interferes

G: Enjoyment of Life

Does Not Interfere ☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 Completely Interferes

Appendix H

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Visual Analogue Scale

☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10

No Pain

Pain as Bad as You Can Imagine

Appendix I

DNA EXTRACTION FROM WHOLE BLOOD FOR THE GENETIC STUDY

Please note that we work in a dedicated space for the Blood DNA extraction - wear appropriate safety gear at all steps of this protocol and use a dedicated set of pipettes.

1. Draw 5ml of blood into an EDTA vacutainer tube (Purple top).
2. Blood can be stored at 4°C up to 1 week before the DNA is extracted.
3. Transfer the blood into a sterile 15ml polypropylene tube.
4. Add 2 volumes (10ml) of TKM1 buffer containing 2.5% NP40.
5. Mix by inverting several times and incubate at room temperature for 10 minutes in order to enhance the haemolysis of the red blood cells.
6. Centrifuge at 3000rpm (1200Xg) at room temperature for 10 minutes.
7. Decant the supernatant leaving the white pellet at the bottom of the tube.
   (Decant in an appropriate blood waste container)
8. Add 1 volume (5ml) of TKM1 buffer (without NP40) and invert and vortex the solution.
9. Centrifuge at 3000rpm (1200Xg) at room temperature for 10 minutes.
10. Decant the supernatant leaving the white pellet at the bottom of the tube.
11. **Repeat steps 7-10 until the pellet in the bottom of the tubes is clean and white.**
12. Add 800µl of TKM2 buffer and 50µl of the 10% SDS solution.
13. Vortex and then mix using a blue pipette tip in order to assist in the lyses of the white blood cells.
14. Incubate for 60 minutes at 55°C in a water bath.
15. Make sure the pellet is totally dissolved before the next steps.
16. Add 150µl of 5M $NaClO_4$ then 500µl of the molecular biology grade chloroform and vortex the solution.
17. Transfer the solution to a sterile 1.5ml microfuge tubes.
18. Centrifuge at 13000rpm at room temperature for 5 minutes.
19. Carefully transfer 500µl of the top aqueous phase to a new sterile microfuge tube.
20. Add 1ml of absolute ethanol and invert until DNA precipitates.
21. Centrifuge at 13000rpm at room temperature for 5-10 minutes
22. Carefully tip off the supernatant leaving the DNA pellet at the bottom of the tube.
23. Allow the DNA pellet to air dry completely and add 200µl of 1X TE buffer.
24. Incubate the tubes at 65°C for 15 min in a heating block. Cool down on ice.
25. Once cooled down, store DNA at -20°C for long-term storage. Avoid freeze thawing cycles and keep at 4°C for short-term storage. Avoid vortexing of DNA to prevent shearing.

REAGENTS

Please have a read through each safety data sheets for each reagent before making your solutions.

Wear the appropriate safety gear at all times. Where necessary, you may need to wear a face mask.

Precautions:

- **EDTA causes serious eye irritation and is harmful if inhaled. Wear protective gloves, protective clothing and face mask to prevent inhalation. It must be used in a well-ventilated area.**
- **SDS is harmful if swallowed, inhaled, causes skin irritation, serious eye damage and respiratory irritation. Keep away from heat/sparks/open flames/hot surfaces. Wear protective gloves, protective clothing and face mask to prevent inhalation.**
- **$NaClO_4$ may cause fire or explosion, it is harmful if swallowed and can cause serious eye irritation. Wear appropriate protective gear.**

TKM1 buffer pH7.6

10mM Tris-HCL

10mM KCl

10mM $MgCl_2$.6H2O

2mM EDTA

Make up 1 Volume which includes 2.5% NP-40 (a detergent) and 1 Volume without NP-40

H_2O to 500ml with autoclaved water in an autoclaved bottle.

Filter sterilise solution with NP-40.

Solution without NP40 can be autoclaved.

TKM2 buffer pH7.6

10mM Tris-HCL

10mM KCl

10mM MgCl2.6H2O

2mM EDTA

0.4 M NaCl

H_2O to 200ml; Autoclave.

10% SDS

Make fresh for best results

20g SDS dissolved in autoclaved H_2O to 200ml

Do not autoclave. Filter sterilise.

1X TE buffer pH 8.0

10mM Tris-HCL

1mM EDTA

H_2O to 100ml; Autoclave.

5M $NaClO_4$

61.2 grams (5M, MW 122.4)

H_2O to 100ml; Autoclave.

Protocol by Lahiri and Nurnberger (1991) with modifications by Mokone et al. (2005).