



This work has been submitted to **NECTAR**, the **Northampton Electronic Collection of Theses and Research**.

Article

Title: Human genetic variation, Sport and Exercise Medicine, and Achilles tendinopathy: role for angiogenesis-associated genes

Creators: Rahim, M., El Khoury, L., Raleigh, S. M., Ribbans, W. J., Posthumus, M., Collins, M. and September, A. V.

DOI: [10.1089/omi.2016.0116](https://doi.org/10.1089/omi.2016.0116)

Example citation: Rahim, M., El Khoury, L., Raleigh, S. M., Ribbans, W. J., Posthumus, M., Collins, M. and September, A. V. (2016) Human genetic variation, Sport and Exercise Medicine, and Achilles tendinopathy: role for angiogenesis-associated genes. *OMICS: A Journal of Integrative Biology*. **20**(9), pp. 520-527. 1536-2310.

It is advisable to refer to the [publisher's version](#) if you intend to cite from this work.

Version: Accepted version

Official URL: <http://online.liebertpub.com/doi/abs/10.1089/omi.2016.0116>

Note: Final publication is available from Mary Ann Liebert, Inc., publishers: <http://dx.doi.org/10.1089/omi.2016.0116>.

<http://nectar.northampton.ac.uk/8883/>





OMICS: A Journal of Integrative Biology: <http://mc.manuscriptcentral.com/omics>

Human Genetic variation, sport and exercise medicine and Achilles tendinopathy: Role for angiogenesis-associated genes

Journal:	<i>OMICS: A Journal of Integrative Biology</i>
Manuscript ID	OMI-2016-0116.R2
Manuscript Type:	Original Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Rahim, Masouda; University of Cape Town Faculty of Health Sciences, Division of Exercise Science and Sports Medicine, Department of Human Biology El Khoury, Louis; University of Essex, School of Biological Sciences Raleigh, Stuart; University of Northampton, Centre for Physical Activity and Chronic Disease, Institute of Health and Wellbeing Ribbans, William; University of Northampton, Centre for Physical Activity and Chronic Disease, Institute of Health and Wellbeing Posthumus, Michael; University of Cape Town Faculty of Health Sciences, Division of Exercise Science and Sports Medicine, Department of Human Biology Collins, Malcolm; University of Cape Town Faculty of Health Sciences, Division of Exercise Science and Sports Medicine, Department of Human Biology September, Alison; University of Cape Town Faculty of Health Sciences, Division of Exercise Science and Sports Medicine, Department of Human Biology
Keyword:	Genetic Variation, Association Study

SCHOLARONE™
Manuscripts

1
2
3
4 **Human genetic variation, sport and exercise medicine, and Achilles**
5 **tendinopathy: Role for angiogenesis-associated genes**
6
7
8
9
10

11
12 Masouda Rahim¹, Louis Y. El Khoury², Stuart M. Raleigh³, William J. Ribbans³, Michael
13 Posthumus¹, Malcolm Collins¹, and Alison V. September¹
14
15
16

17
18
19 ¹Division of Exercise Science and Sports Medicine, Department of Human Biology, Faculty
20 of Health Sciences, University of Cape Town, Cape Town, South Africa;
21
22

23 ²School of Biological Sciences, University of Essex, Colchester, United Kingdom;
24

25 ³Division of Health and Life Sciences, University of Northampton, Northampton, United
26 Kingdom
27
28
29

30
31
32 **Running title:** *Genetics of Achilles Tendinopathy*
33
34
35
36
37

38 **Correspondence:**

39 *Dr. Alison V. September*
40 *Associate Professor*
41 *Division of Exercise Science and Sports Medicine*
42 *University of Cape Town*
43 *PO Box 115, Newlands, 7725*
44 *South Africa*
45

46 Phone: +27 21 650 4559

47 Fax: +27 21 686 7530

48 Email: Alison.September@uct.ac.za
49
50
51
52

53 **Keywords:** Genetic variation; association study; sports medicine; Achilles tendinopathy;
54 vascular endothelial growth factor; kinase insert-domain receptor; polymorphism
55
56
57
58
59
60

1
2
3 ***Contact Information for Co-Authors***
4

5 Masouda Rahim
6

7 Division of Exercise Science and Sports Medicine, University of Cape Town, PO Box 115,
8

9 Newlands, 7725, South Africa
10

11 Phone: +27 21 650 3141
12

13 Fax: +27 21 686 7530
14

15 Email: masouda.rahim@gmail.com
16
17

18
19
20 Louis Y. El-Khoury
21

22 School of Biological Sciences, University of Essex, Colchester, CO4 3SQ, United Kingdom
23

24 Phone: +44 1206 873431
25

26 Email: louis.el-khoury@essex.ac.uk
27
28

29
30 Stuart M. Raleigh
31

32 Centre for Physical Activity and Chronic Disease, Institute of Health and Wellbeing,
33

34 University of Northampton, Boughton Green Road, Northampton, NN2 7AL, United
35

36 Kingdom
37

38 Phone: +44 1604 892306
39

40 Fax: +44 1604 893667
41

42 Email: stuart.raleigh@northampton.ac.uk
43
44

45
46
47 William J. Ribbans
48

49 Centre for Physical Activity and Chronic Disease, Institute of Health and Wellbeing,
50

51 University of Northampton, Boughton Green Road, Northampton, NN2 7AL, United
52

53 Kingdom
54

55 Phone: +44 1604 795415
56
57
58
59
60

1
2
3 Fax: +44 1604 795416
4

5 Email: billribbs@uk-doctors.co.uk
6
7

8
9 Michael Posthumus

10 Division of Exercise Science and Sports Medicine, University of Cape Town, PO Box 115,
11

12 Newlands, 7725, South Africa
13

14
15 Phone: +27 21 650 4573
16

17
18 Fax: +27 21 686 7530
19

20 Email: mposthumus@me.com
21
22

23
24 Malcolm Collins

25 Division of Exercise Science and Sports Medicine, University of Cape Town, PO Box 115,
26

27
28 Newlands, 7725, South Africa
29

30
31 Phone: +27 21 650 6235
32

33
34 Fax: +27 21 686 7530
35

36 Email: malcolm.collins@uct.ac.za
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

List of abbreviations

ACL: anterior cruciate ligament

BMI: body mass index

CON: control group

ECM: extracellular matrix

EDTA: ethylenediaminetetraacetic acid

HWE: Hardy-Weinberg equilibrium

KDR: kinase insert-domain receptor

LD: linkage disequilibrium

RFLP: restriction fragment length polymorphism

PCR: polymerase chain reaction

SA: South Africa

SNP: single nucleotide polymorphism

TEN: Achilles tendinopathy

UK: United Kingdom

VEGFA: vascular endothelial growth factor, A isoform

Abstract

Sport and Exercise Medicine is one of the important subspecialties of 21st century healthcare contributing to improving the physical function, health and vitality of populations while reducing the prevalence of lifestyle-related diseases. Moreover, sport and exercise are associated with injuries such as Achilles tendinopathy, a common tendon injury. The angiogenesis-associated signalling pathway plays a key role in extracellular matrix remodelling, with increased levels of angiogenic cytokines reported after cyclic stretching of tendon fibroblasts. We investigated the variants in angiogenesis genes in relation to the risk of Achilles tendinopathy in two population samples drawn independently from South Africa (SA) and United Kingdom (UK). The study sample was comprised of 120 SA and 130 UK healthy controls, and 108 SA and 87 UK participants with Achilles tendinopathy. All participants were genotyped for five functional polymorphisms in the vascular endothelial growth factor (*VEGFA*) (rs699947, rs1570360, rs2010963) and kinase insert-domain receptor (*KDR*) genes (rs1870377, rs2071559). The *VEGFA* A-G-G inferred haplotype was associated with an increased risk of Achilles tendinopathy in the SA group (15% in controls versus 20% in cases, $p=0.048$) and the combined SA+UK group (14% in controls versus 20% in cases, $p=0.009$). These new findings implicate the *VEGFA* gene with Achilles tendinopathy risk, while highlighting the potential biological significance of the angiogenesis signalling pathway in the aetiology of Achilles tendinopathy. The evidence suggesting a genetic contribution to the susceptibility of sustaining a tendon injury is growing. We anticipate that high-throughput and multi-omics approaches, building on genomics, proteomics and metabolomics, may soon uncover the pathophysiology of many diseases in the field of Sports and Exercise Medicine, as a new frontier of global precision medicine.

Introduction

Sport and Exercise Medicine is one of the important subspecialties of 21st century healthcare contributing to improving the physical function, health and vitality of the population and working together with other disciplines to counter the increasing prevalence of chronic lifestyle-related disease. However, Sport and Exercise Medicine has not received the commensurate attention it deserves in the field of human genetics and the next generation omics technology applications. In this context, Achilles tendinopathy, one of the common soft tissue injuries, is amenable to research using modern genetics approaches. Tendon injuries constitute approximately 30-50% of all sporting injuries (Järvinen et al., 2005), with annual incidence rates of Achilles tendinopathy reported to be between 7-9% for top-level runners (Hirschmüller et al., 2012).

The underlying biological pathways and molecular mechanisms culminating in acute and overuse injuries are currently under investigation in an effort to improve both preventive and treatment modalities. To date, several intrinsic factors, including genetics, have been identified as risk factors predisposing an individual to musculoskeletal soft tissue injuries (Collins and Raleigh, 2009; Meeuwisse, 1994; Posthumus et al., 2011). The results of genetic-association studies, along with histopathological examination and expression studies, have assisted in elucidating some of the key biological pathways that may potentially be involved in the aetiology of musculoskeletal soft tissue injuries (Collins et al., 2015; Riley, 2008; Xu and Murrell, 2008).

Tendons are metabolically active tissues and are able to respond to stimuli but the rate of metabolism is slow (O'Brien, 1997). It is possible that the metabolic rate may increase in response to mechanical loading to allow for remodelling of the extracellular matrix (ECM) (Wang, 2006). Evidence shows that mechanical stimuli causes an increase in the expression of several cell signalling molecules (Jiang et al., 2012; Legerlotz et al., 2012; Skutek et al.,

1
2
3 2001). In particular, a number of studies have reported a pro-angiogenic expression profile
4 after cyclic strain of tenocytes (Mousavizadeh et al., 2014; Nakama et al., 2006; Petersen et
5 al., 2004) and during remodelling (Petersen et al., 2003). Moreover, an increase in vascularity
6 was observed after mechanical loading and in symptomatic Achilles tendons (Lewis et al.,
7 2009; Molloy et al., 2003; Pufe et al., 2001; Scott et al., 2008) whereas normal healthy
8 tendons are relatively avascular structures (Pufe et al., 2001). A number of cell signalling
9 molecules function in the angiogenesis pathway, such as transforming growth factor- β and
10 fibroblast growth factor, with vascular endothelial growth factor (VEGF) considered to be a
11 critical regulator of angiogenesis.
12
13
14
15
16
17
18
19
20
21
22

23 The *VEGFA* gene codes for the A isoform of the protein. This potent angiogenic factor
24 is able to function in a diverse range of signalling pathways (Evans, 2015), and expression
25 levels may be induced by mechanical loading, hypoxia as well as a number of biochemical
26 factors/molecules (Neufeld et al., 1999; Petersen, Pufe, et al., 2003). Although, healthy
27 tendons display negligible amounts of VEGF, this increases significantly during remodelling
28 (Mousavizadeh et al., 2014; Petersen et al., 2004; Pufe et al., 2001). Furthermore, expression
29 levels of VEGF in degenerative tendons are comparable to foetal tendons (Legerlotz et al.,
30 2012; Pufe et al., 2001). VEGF binds to one of two high-affinity receptors, Flt-1 (fms related
31 tyrosine kinase 1) or KDR (kinase insert-domain receptor), localised on the cell surfaces of
32 vascular endothelial cells. KDR, encoded by the *KDR* gene, is considered the major mediator
33 of the angiogenic, mitogenic, and permeability-enhancing effects of VEGF (Bao et al., 2009;
34 Ferrara et al., 2003) both in normal physiology and in pathological processes (Ferrara et al.,
35 2003).
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51

52 Recently, genetic variants within the *VEGFA* and *KDR* genes were associated with risk
53 of anterior cruciate ligament (ACL) ruptures (Rahim et al., 2014). Specifically, independent
54 associations as well as haplotype combinations were implicated, thereby suggesting genomic
55
56
57
58
59
60

1
2
3 intervals harbouring potential DNA motifs within these two genes (Rahim et al., 2014). The
4
5 aim of this study was to investigate the previously associated functional *VEGFA* rs699947,
6
7 rs1570360, rs2010963 and *KDR* rs1870377 and rs2071559 variants with risk of Achilles
8
9 tendinopathy in a South African and a British population.
10

11 12 13 **Materials and methods**

14 15 16 **Participant recruitment**

17
18
19 A total of 120 physically active South African Caucasian asymptomatic control (SA
20
21 CON group) participants and 87 participants with chronic Achilles tendinopathy (SA TEN)
22
23 were included in this study. These samples were previously recruited (2004-2013), according
24
25 to the inclusion and exclusion criteria described by Mokone et al. (Mokone et al., 2005,
26
27 2006). In addition, a previously recruited (2011-2014) British Caucasian study group
28
29 (Rickaby et al., 2015) consisting of 130 asymptomatic controls (UK CON group) and 108
30
31 participants with chronic Achilles tendinopathy (UK TEN) was included. The participants
32
33 from each population were matched for country of birth (self-reported) and provided written
34
35 informed consent. Furthermore, each participant completed questionnaires specifying
36
37 personal details, injury details and medical and sporting history. For the cases, age was
38
39 recorded at time of injury and for the controls at time of recruitment.
40
41
42

43
44 This study was approved by the Faculty of Health Sciences Research Ethics Committee
45
46 within the University of Cape Town, South Africa and the Research Ethics Committee of the
47
48 University of Northampton. This case-control genetic association study is in accordance with
49
50 the recommendations of the STREGA Statement for reporting the results of genetic
51
52 association studies (Little et al., 2009).
53
54
55
56
57
58
59
60

DNA extraction

For the South African participants, approximately 5ml of venous blood was collected from each patient by venipuncture of the forearm vein, into an EDTA Vacutainer[®] tube. Samples were stored at -20°C prior to total DNA extraction using the standard protocol as described by Lahiri and Nurnberger (1991) with slight modifications (Mokone et al., 2006). For the British participants, 2ml of saliva was obtained from each participant for DNA extraction as previously described (Rickaby et al., 2015). DNA extractions were performed at the University of Northampton and the samples made available for this study.

SNP selection and genotyping

The five single nucleotide polymorphisms (SNPs) selected for analysis within the *VEGFA* (rs699947, rs1570360, rs2010963) and *KDR* (rs1870377 and rs2071559) genes were previously implicated with risk of anterior cruciate ligament ruptures (Rahim et al., 2014). All samples (SA group n=273 and UK group n=242) were genotyped for the five polymorphisms using standard genotyping protocols. Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis was used for the *VEGFA* rs699947 (*Bgl*II) (Garza-Veloz et al., 2011) and *KDR* rs1870377 (*Alu*I) SNPs. Details of the primers sequences and PCR conditions are available on request. Restriction fragments were resolved, together with a 100bp size standard, by 2% agarose gel electrophoresis. Catalogued TaqMan[™] Genotyping Assays (Applied Biosystems, Foster City, California, USA) were used for the *VEGFA* rs1570360, *VEGFA* rs2010963 and *KDR* rs2071559 SNPs. The PCR reactions were conducted using the Applied Biosystems StepOnePlus[™] Real-Time PCR system and the Applied Biosystems StepOnePlus[™] Real-Time PCR software v2.2.2 (Applied Biosystems), following manufacturer's recommendations.

1
2
3 Every PCR plate (RFLP and TaqMan™ analyses) contained negative controls (no
4 DNA) and five repeat samples (known genotypes) as a quality control measure for reliable
5 genotyping and detection of contamination. Genotypes were confirmed by two independent
6 investigators, with an average genotyping success rate of 95%. Samples that failed twice to
7 amplify during PCR for a particular SNP were considered unsuccessfully genotyped. (Rahim
8 et al., 2014) All genotyping was conducted at the Division of Exercise Science & Sports
9 Medicine within the University of Cape Town.
10
11
12
13
14
15
16
17
18

19 **Statistical analysis**

20
21 Power calculations were calculated using QUANTO v1.2.4 (<http://hydra.usc.edu/gxe>)
22 to determine sample size for the study. Assuming minor allele frequencies between 0.2 and
23 0.5, a sample size of 85 cases would be adequate to detect an allelic odds ratio (OR) of 2.0
24 and greater at a power of 80% and a significance level of 5%. The programming environment
25 R (R Development Core Team, 2010) was used for all data analysis. Basic descriptive
26 statistics were compared using one-way analysis of variance to determine any significant
27 differences between the characteristics of the CON vs. TEN groups. The R packages *genetics*
28 (Warnes et al., 2011) and *SNPassoc* (González et al., 2007) were used to analyse any
29 differences in genotype and allele frequencies between the diagnostic groups (CON/TEN)
30 and to calculate Hardy-Weinberg equilibrium probabilities and linkage disequilibrium.
31 Haplotypes were inferred using the R package *haplo.stats* (Schaid et al., 2002; Sinnwell and
32 Schaid, 2011). Analysis of the participant characteristics revealed potential confounding
33 variables so for this reason the genotype and haplotype analyses were adjusted accordingly.
34
35 In addition to the analyses within the SA and UK study groups, the genotype and allele
36 frequency distributions were compared between the countries (SA/UK), adjusting for age,
37 weight and diagnostic group and in the combined (SA+UK) groups, adjusting for age, weight
38 and country. Statistical significance was accepted when $p < 0.05$. No adjustments were made
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 for multiple testing as currently no obvious appropriate method exists (Nyholt, 2004;
4
5 Perneger, 1998). The Bonferroni correction was considered too conservative for this study, as
6
7 the tests are on the same group of participants and several of the polymorphisms were in tight
8
9 linkage disequilibrium (Nyholt, 2004). Furthermore, correction for multiple testing was
10
11 considered inappropriate as there was an *a priori* hypothesis (Perneger, 1998; Posthumus et
12
13 al., 2012)
14
15

16 17 **Results**

18 19 **Participant characteristics**

20
21
22
23 The participant characteristics for the SA and UK groups were previously described in
24
25 earlier studies investigating the same study participants (Mokone et al., 2005, 2006; Rickaby
26
27 et al., 2015). Briefly, the SA CON and SA TEN participants were unmatched for age, weight
28
29 and BMI (Table 1). In the UK study group, the CON and TEN participants were matched for
30
31 all characteristics except age (Table 2). There were no significant genotype effects on age,
32
33 sex, height, weight or BMI for any of the polymorphisms (Table S1).
34
35
36

37 38 **Genotype and allele frequencies**

39
40
41 The *VEGFA* rs699947 genotype frequency was significantly different ($p=0.003$)
42
43 between the SA CON and SA TEN groups (Table 3). The CC genotype was significantly
44
45 over-represented in the SA CON group (32%, $n=35$) compared to the SA TEN group (17%,
46
47 $n=14$) ($p=0.019$, OR: 2.30, 95% CI: 1.14-4.64). Additionally, the CA genotype was
48
49 significantly under-represented in the SA CON group (40%, $n=43$) compared to the SA TEN
50
51 group (60%, $n=49$) ($p=0.005$, OR: 2.32, 95% CI: 1.28-4.17). No other significant differences
52
53 in genotype or allele frequency distributions were observed between the CON and TEN
54
55 groups (Table 3) for any of the five polymorphisms investigated in either the South African
56
57
58
59
60

1
2
3 or the British participants. The genotype and minor allele frequency distributions were
4 similar to those reported for other European populations (<http://www.ncbi.nlm.nih.gov/snp/>)
5 and for the previous ACL study by our group (Rahim et al., 2014). All the groups were in
6 HWE except for the UK TEN group for the rs1570360 SNP ($p=0.020$) and the rs1870377
7 SNP ($p=0.015$).
8

9
10
11
12
13
14 Comparisons in genotype and allele frequency distributions between the countries,
15 showed only the *KDR* rs2071559 genotype frequency distribution was significantly different
16 between the groups ($p=0.026$) (Table 3). When the SA+UK groups were combined no
17 significant differences were observed in genotype or allele frequency distribution (Table 3).
18 The linkage disequilibrium (LD) structure of the investigated SNPs within the *VEGFA* gene,
19 is presented in the supplementary material (Fig S1).
20
21
22
23
24
25
26
27

28 **Inferred haplotypes**

29
30
31 Of the eight possible haplotype combinations constructed from the *VEGFA* variants
32 (rs699947 C/A – rs1570360 G/A – rs2010963 G/C), only four (C-G-G, C-G-C, A-G-G, A-A-
33 G) were inferred at a frequency greater than 2%. The A-G-G inferred haplotype combination
34 was significantly under-represented (Fig. 1A) in the SA CON group (15%, $n=18$) vs. the SA
35 TEN group (20%, $n=22$) (dominant model, $p=0.048$; haplo.score:1.97). Similarly, when the
36 SA and UK groups were combined the A-G-G haplotype was significantly under-represented
37 in the SA+UK CON group (14%, $n=35$) vs. the SA+UK TEN group (20%, $n=39$) (dominant
38 model, $p=0.009$; haplo.score:2.61) (Fig. 1C). However, no significant differences were
39 observed between the UK CON and UK TEN groups (Fig. 1B).
40
41
42
43
44
45
46
47
48
49
50

51 Haplotypes were also inferred for the rs2071559 A/G and rs1870377 T/A
52 polymorphisms within the *KDR* gene but no significant differences in frequency distribution
53 were observed between any of the groups after adjusting for the potential confounders (data
54 not shown).
55
56
57
58
59
60

Discussion

The angiogenesis-associated signalling pathway is considered a key biological mediator to promote matrix remodelling. Therefore, this study investigated five polymorphisms within the *VEGFA* (rs699947, rs1570360 and rs2010963) and *KDR* (rs2071559 and rs1870377) genes with risk of Achilles tendinopathy in two independent populations. The main finding of this paper was the haplotype association of the three *VEGFA* SNPs with risk of TEN in the SA group and the combined SA+UK group.

Vascular endothelial growth factor is a potent angiogenic factor, with a key role in the formation of new vasculature (Ferrara et al., 2003). The importance of VEGF in vasculogenesis and angiogenesis was demonstrated by Carmeliet et al. using a mouse gene knockout model, where loss of a single *VEGF* allele led to embryonic lethality (Carmeliet et al., 1996). Consequently, an abundance of research has focused on the role of this growth factor in normal physiology, pathological conditions and as a part of therapeutic strategies. To date, VEGF has been implicated in several pathological conditions including cancer, macular degeneration and rheumatoid arthritis (Evans, 2015). It is hypothesised that angiogenesis also occurs after mechanical loading in an effort to promote remodelling of the tendon extracellular matrix. This is further supported by studies identifying increased VEGF levels after mechanical loading of tenocytes (Mousavizadeh et al., 2014; Nakama et al., 2006; Petersen et al., 2004).

In the present study, the *VEGFA* A-G-G (rs699947 C/A – rs1570360 G/A – rs2010963 G/C) inferred haplotype was associated with increased risk of TEN. This association was noted in both the SA and the combined SA+UK groups which provides further support implicating the *VEGFA* locus in modulating musculoskeletal injury susceptibility (Rahim et al., 2014). The *VEGFA* A-G-G haplotype combination is associated with decreased transcription of the *VEGFA* gene and reduced VEGF plasma levels (Lambrechts et al., 2003).

1
2
3 Hence it is possible that angiogenesis is limited in an already hypovascular structure thus
4 restricting the extent of matrix remodelling that is able to occur. Tang et al. (2016)
5 hypothesised that low VEGF activity may be partly responsible for the slow or weak healing
6 capacity of injured tendons. Furthermore, the authors demonstrated that VEGF gene therapy
7 via an adeno-associated viral type-2 (AAV2) vector resulted in marked improvements in
8 tendon healing and remodelling in an animal model (Tang et al., 2016). Taken collectively,
9 investigation into the role of VEGF in modulating both tendon capacity and injury
10 susceptibility is warranted.
11
12
13
14
15
16
17
18
19

20
21 A recent study implicated the A-A-G inferred haplotype, with decreased risk of non-
22 contact ACL ruptures (Rahim et al., 2014). The explanation for the contrasting haplotype
23 associations observed between TEN and ACL ruptures is not completely understood. It is
24 interesting to note that both these implicated haplotypes are associated with reduced VEGF
25 plasma levels (Lambrechts et al., 2003). One could therefore hypothesise that because the
26 vascularity of tendons and ligaments differ (Benjamin et al., 2006; Fenwick et al., 2002), the
27 biological impact of low levels of VEGF on neovascularisation related to remodelling and
28 capacity would potentially be different between these functionally different tissues.
29 Alternatively, the results may be indicating that these polymorphisms are potentially in
30 linkage with a true risk variant. Thus, it is key that these genomic regions are further
31 explored. Specifically, these associations need to be investigated at a functional level to
32 determine the biological significance of these variants, and of angiogenesis in the repair
33 model for TEN and ACL ruptures.
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48

49
50 It is not surprising that the haplotype implicated in TEN risk modulation is reflected in
51 the independent association of the rs699947 SNP genotype in the South African study group.
52 Moreover, this promoter (-2578C/A) polymorphism is known to be functional, with the C
53 allele correlating to increased VEGF protein production, and the AA genotype linked with
54
55
56
57
58
59
60

1
2
3 lower transcriptional activity (Shahbazi et al., 2002) and increased risk in several phenotypes
4
5 (Andraweera et al., 2012; Lambrechts et al., 2009; Seo et al., 2005). Therefore, there is
6
7 evidence implicating the *VEGFA* gene locus and specific variants at a functional and genetic
8
9 level with risk modulation.
10

11 Although, no independent or haplotype associations were observed for the *KDR* gene,
12
13 this remains a viable candidate for further investigation particularly due to its importance in
14
15 the VEGF signalling pathway. *KDR* is one of two main receptors that *VEGFA* binds to and is
16
17 responsible for the main downstream signalling effects of the ligand (Ferrara et al., 2003).
18
19 Moreover, *KDR* null mutant mice were observed to be embryonically lethal (Shalaby et al.,
20
21 1995). Therefore, it may be worth exploring additional genomic loci within this candidate
22
23 gene as the two polymorphisms (rs2071559 and rs1870377) investigated in the current study
24
25 are situated in the promoter and exon 11, respectively.
26
27
28

29 A limitation of the study was that SA participants were not matched for age, weight or
30
31 BMI. We were unable to determine the weight at time of injury for the cases due to
32
33 incomplete data but several participants reported an increase in weight due to injury-related
34
35 inactivity. Additionally, the older age of the cases may have influenced the weight of the
36
37 cases. In the UK participants, the CON and TEN groups were also unmatched for age and for
38
39 this reason, all statistical analyses were adjusted for the confounders. Although, the UK TEN
40
41 group deviated from HWE at the rs1570360 and rs1870377 loci, the genotype and minor
42
43 allele frequency distributions were similar to those reported for other European populations.
44
45 The main limitation of this study was the small sample size and therefore the SA and UK
46
47 study groups were also combined for the statistical analyses. A minimum sample size of 85
48
49 cases was adequate to detect only allelic odds ratios of 2.0 and greater, whereas the combined
50
51 SA+UK group sample set was sufficient to detect allelic odds ratios of 1.6 and greater.
52
53
54
55
56
57
58
59
60

Conclusions

Our observations collectively suggest that a genomic interval within the *VEGFA* gene was associated with risk of TEN. This gene requires further exploration to understand the potential role of *VEGFA* and specifically angiogenesis in the aetiology of TEN. Investigation into genes involved in the angiogenesis-associated signalling pathway may aid in the development of a risk profile for tendinopathy. In line with this, our future work is aiming towards employing next-generation technologies, specifically whole exome sequencing, to further explore these regions of interest in an effort to further characterise these genomic signatures.

The era of “*precision medicine*” is becoming a large focus underpinning research (Poland et al. 2011; Higdon et al. 2015). The technologies of the *omics* era has revolutionised our capacity to unravel these complex phenotypes. Through Big Data emerging from multi-omics research streams at genomics, proteomics, transcriptomics, epigenomics and bioinformatics levels we can advance the depth and breadth of research in the field of Sports and Exercise Medicine, with a view to improving global health, preventive medicine and quality of life.

Acknowledgements

The authors would like to thank Mr George Mokone and Dr Martin Schwellnus for SA participant recruitment. MR was funded by the University of Cape Town and the National Research Foundation. MP was funded by the Thembakazi Trust. This research was funded in part by the University of Cape Town Research Council, the National Research Foundation and the Department of Science and Technology/ National Research Foundation Collaborative Grant (NRF92550). Authors MC and AS have filed patents on the application of specific

1
2
3 sequence variations (not included in this manuscript) related to risk assessment of anterior
4
5 cruciate ligament ruptures and Achilles tendinopathy.
6
7
8
9

10 **Author disclosure statement**

11
12
13
14 The authors declare no competing financial interests.
15
16
17
18
19

20 **References**

21
22
23
24 Andraweera PH, Dekker GA, Thompson SD, et al. (2012). The interaction between the
25
26 maternal BMI and angiogenic gene polymorphisms associates with the risk of spontaneous
27
28 preterm birth. *Mol Hum Reprod* 18, 459-465.
29
30
31

32
33 Bao P, Kodra A, Tomic-Canic M, et al. (2009). The role of vascular endothelial growth factor
34
35 in wound healing. *J Surg Res* 153, 347–358.
36
37
38

39
40 Benjamin M, Toumi H, Ralphs JR, et al. (2006). Where tendons and ligaments meet bone:
41
42 attachment sites ('entheses') in relation to exercise and/or mechanical load. *J Anat* 208, 471–
43
44 490.
45
46
47

48
49 Carmeliet P, Ferreira V, Brier G, et al. (1996). Abnormal blood vessel development and
50
51 lethality in embryos lacking a single VEGF allele. *Nature* 380, 435–439.
52
53
54

55
56 Collins M and Raleigh SM. (2009). Genetic risk factors for musculoskeletal soft tissue
57
58 injuries. *Med Sport Sci* 54, 136–149.
59
60

1
2
3
4
5 Collins M, September AV and Posthumus M. (2015). Biological variation in musculoskeletal
6
7 injuries: current knowledge, future research and practical implications. *Br J Sports Med* 49,
8
9 1497–1503.
10

11
12
13
14 Evans I. (2015). An Overview of VEGF-Mediated Signal Transduction. In: Fiedler L,
15
16 editor(s). *VEGF Signaling*. Caversham, Reading, UK: Humana Press, p. 91–120.
17
18

19
20
21 Fenwick SA, Hazleman BL and Riley GP. (2002). The vasculature and its role in the
22
23 damaged and healing tendon. *Arthritis Res* 4, 252–260.
24
25

26
27 Ferrara N, Gerber H-P and LeCouter J. (2003). The biology of VEGF and its receptors. *Nat*
28
29 *Med* 9, 669–676.
30
31

32
33
34 Garza-Veloz I, Castruita-De la Rosa C, Cortes-Flores R, et al. (2011). No association
35
36 between polymorphisms/haplotypes of the vascular endothelial growth factor gene and
37
38 preeclampsia. *BMC Pregnancy Childbirth* 11, 35.
39
40

41
42
43 González JR, Armengol L, Solé X, et al. (2007). SNPAssoc: an R package to perform whole
44
45 genome association studies. *Bioinformatics* 23, 644–645.
46
47

48
49 Higdon R, Earl RK, Stanberry L, et al. (2015) The promise of multi-omics and clinical data
50
51 integration to identify and target personalized healthcare approaches in autism spectrum
52
53 disorders. *OMICS* 19(4), 197-208.
54
55

1
2
3 Hirschmüller A, Frey V, Konstantinidis L, et al. (2012). Prognostic value of Achilles tendon
4 Doppler sonography in asymptomatic runners. *Med Sci Sports Exerc* 44, 199–205.
5
6

7
8
9 Järvinen TAH, Kannus P, Maffulli N and Khan KM. (2005). Achilles tendon disorders:
10 Etiology and epidemiology. *Foot Ankle Clin* 10, 255–266.
11
12

13
14
15
16 Jiang C, Shao L, Wang Q and Dong Y. (2012). Repetitive mechanical stretching modulates
17 transforming growth factor- β induced collagen synthesis and apoptosis in human patellar
18 tendon fibroblasts. *Biochem Cell Biol* 90, 667–674.
19
20
21

22
23
24
25 Lahiri DK and Nurnberger JI. (1991). A rapid non-enzymatic method for the preparation of
26 HMW DNA from blood for RFLP studies. *Nucleic Acids Res* 19, 5444.
27
28

29
30
31
32 Lambrechts D, Poesen K, Fernández-Santiago R, et al. (2009). Meta-analysis of vascular
33 endothelial growth factor variations in amyotrophic lateral sclerosis: increased susceptibility
34 in male carriers of the -2578AA genotype. *J Med Genet* 46, 840–846.
35
36
37

38
39
40
41 Lambrechts D, Storkebaum E, Morimoto M, et al. (2003). VEGF is a modifier of
42 amyotrophic lateral sclerosis in mice and humans and protects motoneurons against ischemic
43 death. *Nat Genet* 34, 383–394.
44
45
46

47
48
49
50 Legerlotz K, Jones ER, Screen HRC and Riley GP. (2012). Increased expression of IL-6
51 family members in tendon pathology. *Rheumatology* 51, 1161–1165.
52
53
54

55
56
57
58
59
60
Lewis JS, Raza SA, Pilcher J, et al. (2009). The prevalence of neovascularity in patients

1
2
3 clinically diagnosed with rotator cuff tendinopathy. *BMC Musculoskelet Disord* 10, 163.
4
5

6
7 Little J, Higgins JPT, Ioannidis JPA, et al. (2009). STrengthening the REporting of Genetic
8 Association studies (STREGA) - an extension of the STROBE statement. *Eur J Clin Invest*
9 39, 247–266.
10
11
12

13
14
15
16 Meeuwisse WH. (1994). Assessing Causation in Sport Injury: A Multifactorial Model. *Clin J*
17 *Sport Med* 4, 166–170.
18
19

20
21
22
23 Mokone GG, Gajjar M, September AV, et al. (2005). The guanine-thymine dinucleotide
24 repeat polymorphism within the tenascin-C gene is associated with Achilles tendon injuries.
25
26
27 *Am J Sports Med* 33, 1016–1021.
28
29

30
31
32 Mokone GG, Schwellnus MP, Noakes TD and Collins M. (2006). The COL5A1 gene and
33 Achilles tendon pathology. *Scand J Med Sci Sports* 16, 19–26.
34
35
36

37
38
39 Molloy T, Wang Y and Murrell G. (2003). The roles of growth factors in tendon and
40 ligament healing. *Sports Med* 33, 381–394.
41
42
43

44
45 Mousavizadeh R, Khosravi S, Behzad H, et al. (2014). Cyclic strain alters the expression and
46 release of angiogenic factors by human tendon cells. *PLoS One* 9, e97356.
47
48
49

50
51
52 Nakama LH, King KB, Abrahamsson S and Rempel DM. (2006). VEGF, VEGFR-1, and
53 CTGF cell densities in tendon are increased with cyclical loading: An in vivo tendinopathy
54 model. *J Orthop Res* 24, 393–400.
55
56
57
58
59
60

1
2
3
4
5 Neufeld G, Cohen T, Gengrinovitch S and Poltorak Z. (1999). Vascular endothelial growth
6 factor (VEGF) and its receptors. *FASEB J* 13, 9–22.
7
8

9
10
11 Nyholt DR. (2004). A simple correction for multiple testing for single-nucleotide
12 polymorphisms in linkage disequilibrium with each other. *Am J Hum Genet* 74, 765–769.
13
14
15

16
17
18 O'Brien M. (1997). Structure and metabolism of tendons. *Scand J Med Sci Sports* 7, 55–61.
19

20 Perneger TV. (1998). What's wrong with Bonferroni adjustments. *BMJ* 316, 1236–1238.
21
22
23

24
25 Petersen W, Pufe T, Zantop T, et al. (2003). Hypoxia and PDGF have a synergistic effect that
26 increases the expression of the angiogenetic peptide vascular endothelial growth factor in
27 Achilles tendon fibroblasts. *Arch Orthop Trauma Surg* 123, 485–488.
28
29
30

31
32
33
34 Petersen W, Unterhauser F, Pufe T, et al. (2003). The angiogenic peptide vascular endothelial
35 growth factor (VEGF) is expressed during the remodeling of free tendon grafts in sheep.
36
37
38 *Arch Orthop Trauma Surg.* 123, 168–174.
39
40
41

42
43 Petersen W, Varoga D, Zantop T, et al. (2004). Cyclic strain influences vascular endothelial
44 growth factor (VEGF) and the hypoxia inducible factor 1 alpha (HIF-1a) in tendon fibroblasts.
45
46
47 *J Orthop Res* 22, 847–853.
48
49
50

51
52 Poland GA, Ovsyannikova IG, Kennedy RB, Haralambieva IH, Jacobson RM. (2011)
53
54 Vaccinomics and a new paradigm for the development of preventive vaccines against viral
55
56
57
58
59
60 infections. *OMICS* 15(9), 625-636.

1
2
3
4
5 Posthumus M, Collins M, van der Merwe L, et al. (2012). Matrix metalloproteinase genes on
6 chromosome 11q22 and the risk of anterior cruciate ligament (ACL) rupture. *Scand J Med*
7 *Sci Sport* 22, 523–533.
8
9

10
11
12
13
14 Posthumus M, Collins M, September AV and Schwellnus MP. (2011). The intrinsic risk
15 factors for ACL ruptures: an evidence-based review. *Phys Sportsmed* 39, 62–73.
16
17

18
19
20 Pufe T, Petersen W, Tillmann B and Mentlein R. (2001). The angiogenic peptide vascular
21 endothelial growth factor is expressed in foetal and ruptured tendons. *Virchows Arch* 439,
22 579–585.
23
24
25
26

27
28
29 R Development Core Team. (2010). R: a language and environment for statistical computing.
30 R Foundation for Statistical Computing, www.r-project.org.
31
32

33
34
35
36 Rahim M, Gibbon A, Hobbs H, et al. (2014). The association of genes involved in the
37 angiogenesis-associated signaling pathway with risk of anterior cruciate ligament rupture. *J*
38 *Orthop Res* 32, 1612–1618.
39
40
41

42
43
44
45 Rickaby R, El Khoury L, Ribbans WJ and Raleigh SM. (2015). Variation within three
46 apoptosis associated genes as potential risk factors for Achilles tendinopathy in a British
47 based case–control cohort . *Gene* 571, 167–171.
48
49
50

51
52
53
54 Riley G. (2008). Tendinopathy-from basic science to treatment. *Nat Clin Pract Rheumatol* 4,
55 82–89.
56
57
58
59
60

1
2
3
4
5 Schaid DJ, Rowland CM, Tines DE, et al. (2002). Score tests for association between traits
6 and haplotypes when linkage phase is ambiguous. *Am J Hum Genet* 70, 425–434.
7
8

9
10
11 Scott A, Lian Ø, Bahr R, et al. (2008). VEGF expression in patellar tendinopathy: a
12 preliminary study. *Clin Orthop Relat Res* 466, 1598–1604.
13
14
15

16
17
18 Seo JS, Lee S-S, Kim SUIY, et al. (2005). Influence of VEGF gene polymorphisms on the
19 severity of ankylosing spondylitis. *Rheumatology (Oxford)* 44, 1299–1302.
20
21
22

23
24
25 Shahbazi M, Fryer AA, Pravica V, et al. (2002). Vascular endothelial growth factor gene
26 polymorphisms are associated with acute renal allograft rejection. *J Am Soc Nephrol* 13,
27 260–264.
28
29
30

31
32
33 Shalaby F, Rossant J, Yamaguchi TP, et al. (1995). Failure of blood-island formation and
34 vasculogenesis in Flk-1-deficient mice. *Nature* 376, 62–66.
35
36
37

38
39
40 Sinnwell J and Schaid D. (2011). Haplo.stats: a package for statistical analysis of haplotypes
41 with traits and covariates when linkage phase is ambiguous. R package (version 1.5.4).
42
43
44

45
46
47 Skutek M, van Griensven M, Zeichen J, et al. (2001). Cyclic mechanical stretching enhances
48 secretion of Interleukin 6 in human tendon fibroblasts. *Knee Surg Sports Traumatol Arthrosc*
49 9, 322–326.
50
51
52

53
54
55 Tang JB, Wu YF, Cao Y, et al. (2016). Basic FGF or VEGF gene therapy corrects
56
57
58
59
60

1
2
3 insufficiency in the intrinsic healing capacity of tendons. *Sci Rep* 6, 20643.
4
5

6
7 Wang JHC. (2006). *Mechanobiology of tendon*. *J Biomech* 39, 1563–1582.
8
9

10
11 Warnes G, Gorjanc G, Leisch F and Al. E. (2011). *Genetics: a package for population*
12
13 *genetics*. R Package (version 1.3.6).
14
15

16
17
18 Xu Y and Murrell GAC. (2008). *The basic science of tendinopathy*. *Clin Orthop Relat Res*
19
20 466, 1528–1538.
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table 1: Characteristics of the South African (SA) asymptomatic control (CON) group and the Achilles tendinopathy (TEN) group.

	SA CON (<i>n</i> = 120)	SA TEN (<i>n</i> = 108)	p-value ^a
Age* (years)	37.3 ± 10.4 (115)	42.9 ± 13.6 (89)	0.001
Sex (% male)	63.3 (120)	70.5 (105)	0.294
Height (cm)	175.0 ± 9.5 (116)	176.5 ± 8.7 (93)	0.239
Weight (kg)	71.4 ± 12.1 (119)	78.8 ± 18.2 (98)	<0.001 (0.023‡)
Body mass index (kg/m ²)	23.3 ± 2.7 (116)	25.3 ± 4.3 (93)	<0.001
Country of birth (% South African)	76.3 (118)	79.8 (104)	0.526

Values are expressed as mean ± standard deviation; sex and country of birth are represented as a percentage.

The number of participants (*n*) with available data for each variable is in parentheses.

* Age shows self-reported values at the time of onset of symptoms for the TEN group and at time of recruitment for the CON group

^a CON vs. TEN, p-values in bold typeset indicates significance (*p*<0.05)

‡ p-value adjusted for age and sex

Table 2: Characteristics of the British (UK) asymptomatic control (CON) group and the Achilles tendinopathy (TEN) group.

	UK CON (<i>n</i> = 130)	UK TEN (<i>n</i> = 87)	p-value ^a
Age* (years)	41.6 ± 11.6 (123)	45.2 ± 14.4 (87)	0.044
Sex (% male)	63.1 (130)	58.6 (87)	0.509
Height (cm)	174.9 ± 10.4 (123)	172.3 ± 8.5 (84)	0.057
Weight* (kg)	80.2 ± 19.5 (124)	77.1 ± 15.0 (62)	0.263
Body mass index* (kg/m ²)	25.9 ± 4.5 (123)	25.9 ± 4.1 (62)	0.983
Country of birth (% British)	96.0 (124)	96.5 (86)	1.000

Values are expressed as mean ± standard deviation; sex and country of birth are represented as a percentage.

The number of participants (n) with available data for each variable is in parentheses.

* Age, weight and body mass index (BMI) are self-reported values at the time of onset of symptoms for the TEN group and at time of recruitment for the CON group

^a CON vs. TEN, p-values in bold typeset indicates significance (p<0.05)

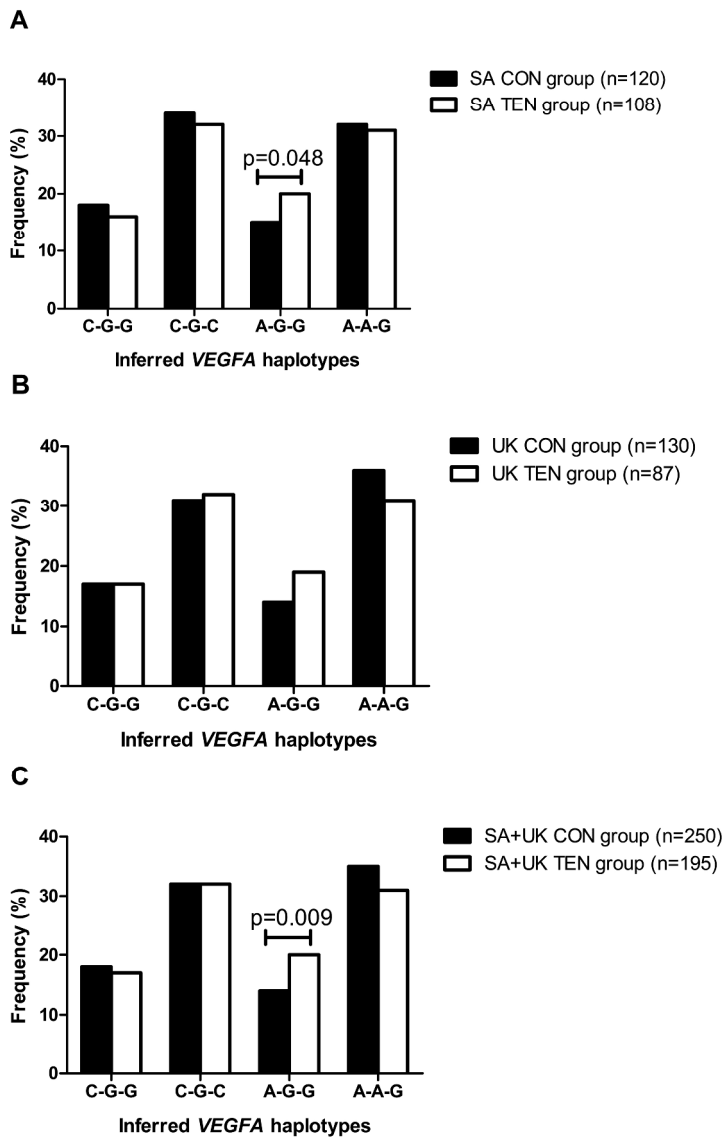
Table 3: Genotype and minor allele frequency distributions, and p-values for Hardy-Weinberg exact test of the polymorphisms investigated in the *VEGFA* and *KDR* genes in all participants (male and female) in the control (CON) and the Achilles tendinopathy (TEN) groups within the South African (SA) and British (UK) populations.

		South African (SA)		British (UK)		p-values			
		CON	TEN	CON	TEN	Country*	Diagnostic group		
							SA*	UK†	SA+UK*
<i>VEGFA</i> rs699947	n	108	81	121	85				
	CC	32 (35)	17 (14)	28 (34)	25 (21)	0.456	0.003	0.864	0.051
	CA	40 (43)	61 (49)	46 (55)	51 (43)				
	AA	28 (30)	22 (18)	26 (32)	25 (21)				
	A allele	48 (103)	52 (85)	49 (119)	50 (85)	0.777	0.357	0.869	0.594
	HWE	0.089	0.117	0.380	1.000				
<i>VEGFA</i> rs1570360	n	105	75	111	82				
	GG	44 (46)	44 (34)	40 (44)	52 (43)	0.162	0.788	0.178	0.564
	GA	45 (47)	47 (37)	42 (47)	32 (26)				
	AA	11 (12)	9 (7)	18 (20)	16 (13)				
	A allele	34 (71)	33 (51)	39 (87)	32 (52)	0.423	0.970	0.130	0.193
	HWE	1.000	0.651	0.242	0.020				
<i>VEGFA</i> rs2010963	n	114	82	118	85				
	GG	42 (47)	42 (34)	49 (58)	46 (39)	0.778	0.883	0.863	0.733
	GC	49 (56)	50 (41)	38 (45)	42 (36)				
	CC	10 (11)	9 (7)	13 (15)	12 (10)				
	C allele	34 (78)	34 (55)	32 (75)	33 (56)	0.636	0.889	0.805	0.942
	HWE	0.412	0.506	0.217	0.806				
<i>KDR</i> rs2071559	n	115	83	119	85				
	GG	35 (40)	28 (23)	26 (31)	22 (19)	0.026	0.438	0.752	0.425
	GA	42 (48)	43 (36)	52 (62)	55 (47)				
	AA	24 (27)	29 (24)	22 (26)	22 (19)				
	A allele	44 (102)	51 (84)	48 (114)	50 (85)	0.528	0.218	0.676	0.347
	HWE	0.139	0.181	0.475	0.391				
<i>KDR</i> rs1870377	n	108	79	116	82				
	TT	56 (60)	51 (40)	62 (72)	57 (47)	0.475	0.693	0.184	0.689
	TA	38 (41)	43 (34)	32 (37)	29 (24)				
	AA	7 (7)	6 (5)	6 (7)	13 (11)				
	A allele	25 (55)	28 (44)	22 (51)	28 (46)	0.619	0.606	0.167	0.144
	HWE	1.000	0.467	0.436	0.015				

Genotype and allele frequencies are expressed as a percentage with the number of participants (n) in parentheses. p-values are for differences in genotype and minor allele

1
2
3 frequencies between countries (SA/UK) and diagnostic groups (CON/TEN), adjusted for one
4 another. Differences between the diagnostic groups in the South African, British and
5 combined (SA+UK) groups are shown in the table. * indicates p-values are adjusted for age
6 and weight. † indicates p-values are adjusted for age and bold typeset indicates significance
7 ($p < 0.05$) p-values for the exact test of Hardy-Weinberg equilibrium (HWE) for each of the
8 groups are included in the table.
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



129x197mm (600 x 600 DPI)

tribution

1
2
3 **Figure 1:** Inferred haplotype frequency distributions for the *VEGFA* rs699947, rs1570360
4 and rs2010963 polymorphisms in the control group (CON; black bars) and the Achilles
5 tendinopathy group (TEN; white bars) for (A) the South African (SA) participants, (B) the
6 British (UK) participants and (C) the combined SA and UK participants. Statistically
7 significant differences in haplotype frequency between the groups are depicted on the graph,
8 with p-values adjusted for age and weight. The number of participants (n) in each group is in
9 parentheses.
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Supplementary data

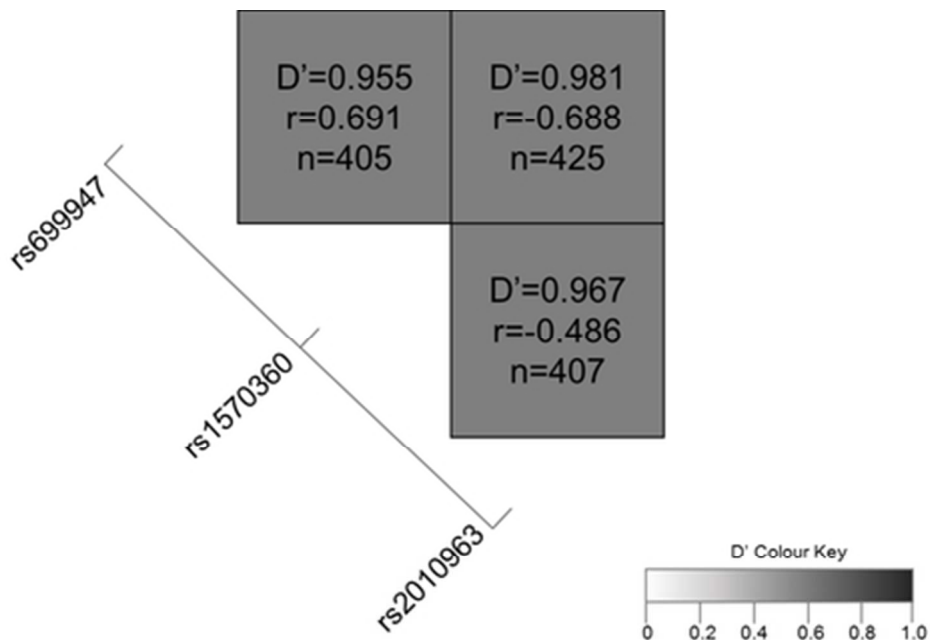
Table S1: Genotype effects on descriptive measures for the *VEGFA* rs699947, *VEGFA* rs1570360, *VEGFA* rs2010963, *KDR* rs2071559 and *KDR* rs1870377 polymorphisms in the South African (SA) and British (UK) Achilles tendinopathy groups

	<i>VEGFA</i> rs699947		<i>VEGFA</i> rs1570360		<i>VEGFA</i> rs2010963		<i>KDR</i> rs2071559		<i>KDR</i> rs1870377	
	<i>SA</i>	<i>UK</i>	<i>SA</i>	<i>UK</i>	<i>SA</i>	<i>UK</i>	<i>SA</i>	<i>UK</i>	<i>SA</i>	<i>UK</i>
Age	0.799	0.227	0.706	0.405	0.473	0.857	0.706	0.553	0.323	0.894
Sex	0.080	0.540	0.652	0.472	0.506	0.139	0.836	0.868	0.489	0.687
Height	0.020	0.883	0.953	0.619	0.102	0.361	0.685	0.644	0.119	0.805
Weight	0.062	0.126	0.624	0.608	0.717	0.599	0.727	0.265	0.547	0.241
BMI	0.595	0.088	0.464	0.611	0.982	0.120	0.568	0.136	0.980	0.196

Values expressed are p-values adjusted for group, critical age, sex, critical body mass index (BMI).

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

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



20x13mm (600 x 600 DPI)

1
2
3 **Figure S1:** Linkage disequilibrium (LD) structure mapping across the three *VEGFA* SNPs
4 (rs699947, rs1570360 and rs2010963) on chromosome 6p12. The LD values (D') were
5 calculated using the combined genotyping data of the CON and TEN groups for the South
6 African (SA) and British (UK) study groups. The D' values between pairs of markers are in
7 each cell and the strength of the linkage is indicated by the cell colour, corresponding to the
8 colour key. The cells depict the D' values, sample size (n) and the correlation coefficient (r)
9 for each association.
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60