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# Polymorphic variation within the *ADAMTS2*, *ADAMTS14*, *ADAMTS5*, *ADAM12* and *TIMP2* genes and the risk of Achilles tendon pathology: A genetic association study

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ABSTRACT

*Objectives:* Achilles tendon pathology (ATP) is a multifactorial condition for which genetic risk factors have been identified. The *ADAMTS*, *ADAM12* and *TIMP2* genes encode enzymes that are important regulators of tendon homeostasis. ADAMTS2 and ADAMTS14 proteins are procollagen N-propeptidases for procollagen type I, type II, and type III. *ADAMTS2*, like *COL5A1*, has been linked to Ehlers–Danlos syndrome. Variants within *ADAMTS5* and *ADAM12* have been associated with osteoarthritis. TIMP2, a metalloprotease inhibitor, maintains homeostasis in the ECM by inhibiting ADAM, ADAMTS and MMP functions. We sought to determine whether single nucleotide polymorphisms (SNPs) within the *ADAMTS2*, *ADAMTS5*, *ADAMTS14*, *ADAM12* and *TIMP2* genes were associated with the risk of ATP in two independent populations.
*Design:* 213 (115 ATP cases and 98 asymptomatic controls) South African Caucasian participants and 209 (60 ATP cases and 149 asymptomatic controls) Australian Caucasian participants were recruited for this case–control genetic association study.
*Methods:* All participants were genotyped using TaqMan technology for the *ADAMTS2* rs1054480, *ADAMTS5* rs226794, *ADAMTS14* rs4747096, *ADAM12* rs3740199, and *TIMP2* rs4789932 SNPs.
*Results:* We report for the first time a significant ($p = 0.016$) genotypic association between the *TIMP2* rs4789932 variant and ATP in a combined Caucasian cohort. We also identify an interaction between the *ADAMTS14* rs4747096 variant and age of onset of ATP ($p = 0.024$).
*Conclusions:* Our data show that DNA sequence variation within the *TIMP2* gene is a risk factor for ATP in Caucasians. Furthermore, carriage of the *ADAMTS14* rs4747096 GG variant appears to delay onset of the injury in the ATP group.


## 1. Introduction

Achilles tendon pathology (ATP), consisting of chronic Achilles tendinopathy and acute Achilles tendon ruptures, typically occurs as a result of acute or repetitive mechanical loading during occupational and sporting activities.[1] Although the exact underlying aetiology of ATP remains to be defined, a number of intrinsic (including genetic) and extrinsic risk factors have been identified.[2]

Previous studies have shown that variants within the *TNC*,[3] *COL5A1*,[4,5] *MMP3*,[6] *GDF5*,[7] and *CASP8* genes[8] independently associate with the risk of ATP. Since ATP is a multifactorial condition, its development is likely to have a complex genetic component and additional candidate genes should be investigated.[1]

All genes which have been shown to associate with Achilles tendinopathy to date encode for proteins that are either structural and/or regulatory in function.[1] Additional genes encoding for extracellular matrix (ECM) specific proteinases and their inhibitors are therefore good candidates for further investigation. In addition to the MMP family of proteins, the ADAM (a disintegrin and metalloproteinase), ADAMTS (a disintegrin and metalloproteinase

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with thrombospondin motifs), and the TIMP (tissue inhibitor metalloproteinase) family of proteins are all involved in the regulation of the composition of the ECM of tendon and other connective tissues.[9] Changes in the composition of the extracellular matrix of tendon are likely to be reflected in the biomechanical properties of this connective tissue.

The ADAM family of trans-membrane proteins belong to the super-family of zinc proteases.[10] There are 19 different ADAM genes, which are involved in various functions such as: cell–cell interaction, fertilisation, and muscle development.[10] Interestingly, the expression of ADAM12 and variation within the ADAM12 gene have previously been associated with Achilles tendinopathy[11] and osteoarthritis.[12]

In addition to the ADAM family, the ADAMTS proteinases are a group of enzymes known to influence development, angiogenesis, coagulation, as well as maintaining homeostasis in the ECM.[9] ADAMTS2, a major tendon procollagen N-propeptidase, is reported to be highly expressed in pathologic compared with healthy tendons.[11] Furthermore, mutations within the *ADAMTS2* gene have been shown to be associated with connective tissue disorders such as Ehlers–Danlos syndrome VIIC.[13] ADAMTS14 is a homologue of ADAMTS2[14] and a major type I Procollagen N-propeptidase in tendons.[9,11] The *ADAMTS14* rs4747096 variant, a putative deleterious non-synonymous single nucleotide polymorphism (SNP), has been associated with osteoarthritis.[15] The *ADAMTS2* rs1054480, a non-synonymous SNP, is also is predicted to be deleterious.[15,16]

Previous studies have reported a decrease in the expression level of ADAMTS5, in Achilles tendinopanthy.[11] ADAMTS5 has also been implicated in osteoarthritis.[17] Although genetic variation in the *ADAMTS5* gene has not been associated with osteoarthritis, a putative deleterious non-synonymous variant, within the gene (rs226794), showed a tendency to be over-represented within European Caucasians with osteoarthritis.[18]

The *TIMP* (tissue inhibitor metalloproteinase) genes encode proteins that inhibit the action of MMPs, ADAMs and ADAMTSs.[10] Elevated expression of TIMP2 RNA has previously been found in ruptured Achilles tendon samples compared to healthy controls.[19] In contrast an earlier study reported lower levels of TIMP2 RNA in ruptured Achilles tendon relative to healthy control tissue.[11] In addition to RNA it has been shown that serum TIMP2 protein remains high even as long as three years post Achilles tendon injury.[20] Interestingly, the rs4789932 variant within the *TIMP2* promoter has also been investigated for its role in the development of different cancers.[21]

From the preceding paragraphs it seems that genomic variation within each of the genes discussed may have relevance as a predisposing factor for injury to the Achilles tendon. We base this hypothesis on the fact that each gene has an important role in the integrity of the extracellular matrix and that alteration within the gene sequence or level of expression has in some cases been shown to be associated with musculoskeletal soft tissue pathology. Accordingly, the aims of this study were to determine whether the *ADAMTS2* rs1054480, *ADAMTS5* rs226794, *ADAMTS14 rs4747096*, *ADAM12* rs3740199, and *TIMP2* rs4789932 variants were associated with ATP in two geographically distinct Caucasian populations.

## 2. Methods

One hundred and seventy-three (59 Australian (AUS) and 114 South African (SA)) self-reported Caucasian participants diagnosed with Achilles tendon pathology (ATP) and 248 (152 AUS and 96 SA) asymptomatic Caucasian controls (CON) were recruited for this case–control genetic association study as previously described.[4,5] Participants gave written informed consent according to the Declaration of Helsinki and completed a medical and injury history questionnaire. The Research Ethics Committees of the Faculty of Health Sciences at the University of Cape Town, South Africa, La Trobe University, Australia, Monash University, Australia and the University of Northampton, United Kingdom approved this study.

The ATP group consisting of 134 participants diagnosed with chronic Achilles tendinopathy (59 from AUS and 75 from SA) and 39 participants with complete or partial Achilles tendon rupture (all from SA). Chronic Achilles tendinopathy was diagnosed using clinical criteria as previously described.[4,5] In all the AUS and 63 of 114 SA participants, soft tissue ultrasound examination was performed to confirm the diagnosis of the affected Achilles tendon.[4,5] The diagnosis of Achilles tendon rupture was made clinically using standard validated criteria and confirmed in all cases by examination at the time of surgery and/or by imaging as previously described.[4]

For the Australian cohort, DNA was extracted from whole blood using a sequence extraction technique (Flexigene DNA kit, Qiagen P/L, Valencia, CA, USA) as per the manufacturer's recommendations. DNA for the South African cohort was extracted from whole blood using the procedure described by Lahiri and Nurnberg[22] and modified by Mokone et al.[3,4]

All participants were genotyped for the *ADAMTS2* rs1054480, *ADAMTS5* rs226794, *ADAMTS14* rs4747096, *ADAM12* rs3740199, and *TIMP2* rs4789932 gene variants using fluorescence-based TaqMan assays (Applied Biosystems, Foster City, CA, USA). The assay used for *ADAMTS2* rs1054480 was selected from the Applied Biosystems pre-designed human assays selection, whereas the primers and probes for the remaining variants were custom-designed Taqman assays (Applied Biosystems, Foster City, CA, USA). Each PCR reaction contained probes and primers in a PCR mastermix containing AmpliTaq DNA Polymerase Gold (Applied Biosystems, Foster City, CA, USA) in a reaction volume of 12 μL. PCR was performed on an Applied Biosystems StepOnePlus™ Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). Genotypes were automatically called using Applied Biosystems StepOnePlus™ real-time PCR software Version 2.1 (Applied Biosystems, Foster City, CA, USA). Rox was used as a passive reference and each PCR run included both positive (known genotype) and negative (water) controls for quality control.

Data were analysed using STATISTICA Version 10.0 (Statsoft Inc., Tulsa, OK, USA) and Graphpad InStat Version 5 (Graphpad Software, San Diego, CA, USA) statistical programmes (except 'program' in computers). Sample size for this study has been previously calculated.[3,4] A one-way analysis of variance was used to determine any significant differences between the characteristics of the ATP and CON groups within the Australian and South African cohorts. A Chi-squared ($\chi^2$) analysis or Fisher's exact test was used to analyse any differences in the genotype and allele frequencies, as well as other categorical data between the groups. Significance was accepted when $p < 0.05$. Hardy–Weinberg equilibrium was established using the programme (except 'program' in computers) Genepop web version 3.4 (http://genepop.curtin.edu.au/).

## 3. Results

The SA CON, AUS CON, SA ATP and AUS ATP groups were similarly matched for age and country of birth (Table 1). There were however significantly more females in the AUS CON group ($p < 0.001$) when compared to the other three groups. When co-varied for sex, the four groups were similarly matched for height. The AUS ATP and SA ATP groups were recruited on average $8.8 \pm 10.0$ (58) and $8.0 \pm 9.0$ (107) years, respectively, after their initial injury. Even when co-varied for sex and age of recruitment both the AUS and SA TEN groups were significantly heavier ($p < 0.001$)