The *COL5A1* gene and musculoskeletal soft-tissue injuries

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Abstract

Background. It has been shown that there is an association between various genetic variants and Achilles tendon injuries as well as anterior cruciate ligament (ACL) ruptures. Among other variants the *Bst*UI restriction fragment length polymorphism (RFLP) within the *COL5A1* gene has been shown to be over-represented in asymptomatic participants when compared with those with chronic Achilles tendinopathy, and in asymptomatic female participants when compared with those with ACL ruptures. The male asymptomatic control participants in the ACL study, which were 10 years younger than previously investigated cohorts, had a distinctly different genotype frequency.

Aim. The aim of this study was therefore to determine whether the distribution of the *COL5A1 Bst*UI RFLP in the combined asymptomatic participants without any known history of tendon injuries is age dependent, particularly among males.

Results. When the 265 male asymptomatic participants from all studies were pooled and divided into age-group tertiles, there was a significant linear increase in the CC genotype frequency (p=0.032) among the male age groups, with the youngest group having the lowest frequency (CC genotype frequency, 13%) and the oldest group having the highest (CC genotype frequency, 27%) frequency. There was however a similar CC genotype content in all three female (N=231) age groups (CC genotype frequency, 24 - 27%; p=0.795).

Conclusion. The practical implication is that the selection of asymptomatic groups is of critical importance when future studies of this nature are designed. Future research investigating this genetic variant as a risk factor for soft-tissue injuries should consider these findings when selecting asymptomatic participants.

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Introduction

Acute and chronic musculoskeletal soft-tissue injuries are common during participation in physical activity.[1] Multiple extrinsic and intrinsic risk factors are implicated in the aetiology of these complex injuries.[2,3] In two specific injuries, anterior cruciate ligament (ACL) ruptures and chronic Achilles tendinopathy, genetic components have been identified as intrinsic risk factors. Among the genetic risk factors identified, the *CO5A1 Bst*UI restriction fragment length polymorphism (RFLP) has been associated with both chronic Achilles tendinopathy and ACL ruptures.[3-5]

The CC genotype of the *COL5A1 Bst*UI RFLP was significantly over-represented in asymptomatic participants compared with those with chronic Achilles tendinopathy – both in South African[4] and Australian[3] populations. A similar finding was reported when female participants with ACL ruptures were compared with asymptomatic female controls.[5] All control groups in these studies were matched for physical activity and physiological characteristics. These data suggest that individuals with a CC genotype are protected, despite the particular load and/or external forces applied to their musculoskeletal soft tissues. However, the CC genotype of the *COL5A1 Bst*UI RFLP was not over-represented in male subjects with ACL ruptures compared with asymptomatic male controls.[5] Owing to the reported increased risk of ACL ruptures among females, the ACL study analysed males and females separately. The previous two Achilles tendinopathy studies only analysed males and females as one group.

Interestingly, the CC genotype frequency of the male asymptomatic participants of the ACL study was distinctly lower than the CC genotype frequencies of the asymptomatic control cohorts in which the CC genotype was over-represented.[3-5] Furthermore, the male asymptomatic participants of the ACL study were approximately 10 years younger than the asymptomatic control groups in the previous two Achilles tendinopathy studies.

Table I. The general physiological characteristics of the male and female age group tertiles

	≤25-year group	26 - 42-year group	≥43-year group	*p*-value
Males	*N*=83	*N*=112	*N*=70	
Age (yrs)	22.3±1.7[*†]	33.0±5.0[*‡]	52.6±8.2[†‡]	
	(18 - 25)	(26 - 42)	(43 - 77)	<0.001
Height (cm)	180±6	180±6	178±7	
	(168 - 195)	(167 - 201)	(164 - 195)	0.590
Weight (kg)	79.9±11.5	81.4±12.2	81.9±15.0	
	(61.7 - 110.0)	(59.7 - 137.0)	(58.0 - 136.0)	0.065
BMI (kg/m^2)	24.5±2.8[§]	25.2±3.4	26.0±4.3[§]	
	(18.5 - 31.7)	(19.9 - 38.4)	(20.2 - 39.3)	0.036
South African born (%)	79.5[*†]	47.7[*]	40.5[†]	<0.001
Australian born (%)	10.3	29.4	36.2	
Females	N=63	N=108	*N*=60	
Age (yrs)	22.6±1.7[*†]	32.4±4.5[*‡]	52.0±6.7[†‡]	
	(19 - 25)	(26 - 42)	(43 - 72)	<0.001
Height (cm)	166±7	168±7[‡]	164±8[‡]	
	(152 - 179)	(152 - 187)	(145 - 181)	0.004
Weight (kg)	61.2±6.5	64.4±9.0	81.9±15.0	
	(49.0 - 79.2)	(48.0 - 87.0)	(47.0 - 115.0)	0.051
BMI (kg/m^2)	22.1±2.1[†]	22.9±3.0	24.1±4.6[†]	
	(18.1 - 28.1)	(18.1 - 33.2)	(18.6 - 46.7)	0.005
South African born (%)	66.1[*†]	31.5[*]	21.7[†]	<0.001
Australian born (%)	25.4	48.2	56.7	

Values are expressed as mean ± standard deviation with the range in parentheses or as a frequency. The number (*N*) of male and female participants in each age group is also indicated.
BMI – body mass index.
Post-hoc analysis: [*]<0.001; [†]<0.003; [‡]≤0.002; [§]=0.026.

The objective of this study was therefore to further examine the age- and sex-related changes in the *COL5A1 Bst*UI RFLP genotype frequency among the combined asymptomatic participants. More specifically, our primary aim was to determine if the CC genotype frequency of the *COL5A1 Bst*UI RFLP among male and female subjects without a previous history of tendon injury is age dependent.

Methods

All 496 asymptomatic participants (265 male and 231 female) without a reported history of tendon injuries that were previously investigated in three separate publications were included in this analysis.[3-5] Because of the design of the previous tendinopathy studies, it was not possible to exclude those with a history of ligament injuries. Prior to participation in these original studies, all participants gave written informed consent and completed a medical history questionnaire form. All descriptive data for the subjects with Achilles tendinopathy, as well as the asymptomatic control groups, were previously reported.[3-5] All three studies were approved by the Research Ethics Committee of the Faculty of Health Sciences of the University of Cape Town and/or the Human Ethics Committee of La Trobe University, Melbourne, Australia.

The participants were combined and divided into three male and three female age groups: (*i*) ≤25 years old; (*ii*) 26 - 42 years old; and (*iii*) ≥43 years old. A chi-squared (χ^2) test, Fisher's exact test or χ^2 test for linear trends was used to analyse differences in genotype and any other categorical data between the groups. Data were analysed using STATISTICA Version 8.0 (Statsoft Inc., Tulsa, Oklahoma, USA) and Graphpad InStat Version 3 (Graphpad Software, San Diego, California, USA) statistical programs. Statistical significance was accepted when $p<0.05$. A one-way analysis of variance (ANOVA) was used to determine any significant difference between the characteristics of the male and female age groups. Hardy-Weinberg equilibrium values were established using the program Genepop web version 3.4 (http://genepop.curtin.edu.au/).

Results

There was a significant linear trend ($p=0.032$) for the CC genotype frequency among the male age groups (Fig. 1A). The youngest group had the lowest CC frequency (13%), and the oldest group the highest CC frequency (27%) (Fig. 1A). The CC genotype content in all three female age groups (24 - 27%) was similar ($p=0.795$, Fig. 1B). The general characteristics of the male and female age group tertiles are described in Table I.

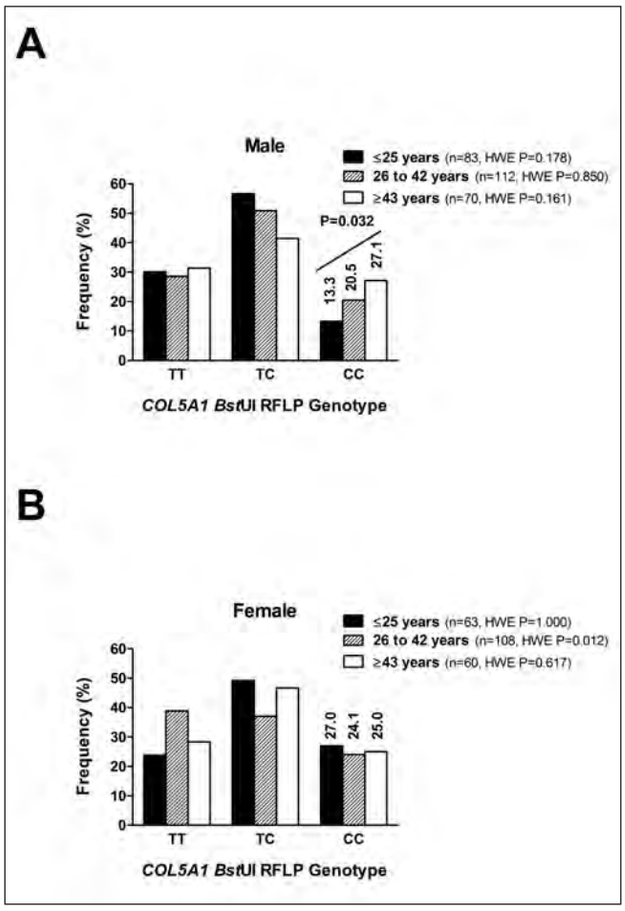

Fig. 1. The genotype frequency of the* COL5A1 Bst*UI restriction fragment length polymorphism (RFLP) in all (A) male and (B) female asymptomatic participants divided according to age into participants (≤25 years old black bars), 26 - 42 years old (thatched bars), and >42 years old (clear bars). A significant linear trend (p=0.032) for the CC genotype content among the male age groups was found. The number of subjects (N) within each category, as well as the Hardy-Weinberg equilibrium (HWE) p-values, are shown in parentheses.

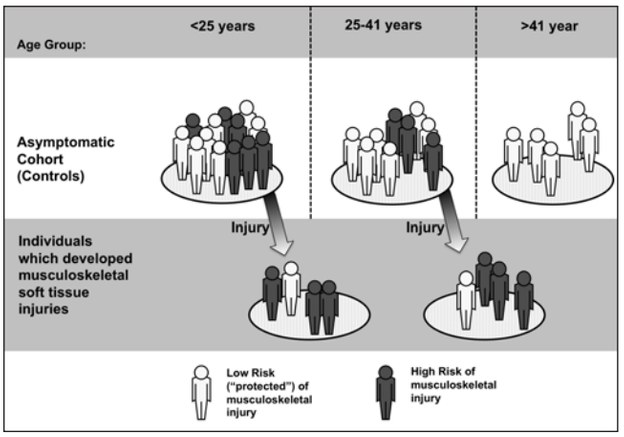

Fig. 2. Proposed explanation for the significant linear trend in CC genotype frequency among the asymptomatic male subjects when divided into the three age groups (<25 years, 25 - 41 years, and >41 years). It is proposed that asymptomatic subjects in the age category <25 years will more than likely consist of individuals at high (black shaded) and low (no shade) risk of musculoskeletal soft-tissue injury. Among older asymptomatic groups of participants (25 - 41 years, and >41 years) the relative proportion of individuals at high risk of injury will be reduced, as the likelihood of high-risk individuals becoming injured over time is greater than the likelihood of low-risk individuals becoming injured.

Discussion

The main finding of this study was that there is a significant age-dependent increase in the distribution of the *COL5A1 Bst*UI RFLP CC genotype in the pooled asymptomatic male participants of the three studies which investigated this polymorphism as a possible risk factor for musculoskeletal soft-tissue injuries. No similar trends were observed in the female subjects.

We propose that the reported finding indicates that the youngest group of asymptomatic male participants consists of a mixture of individuals, similar to the general population, who are at low and high risk of musculoskeletal soft-tissue injuries (Fig. 2). However, when older subjects (who would have had a greater amount of exposure to extrinsic factors) are analysed, individuals who may have been previously uninjured, would have developed an injury. Therefore, when older asymptomatic participants are analysed, the group will contain a highly selected sample of the population at low risk of musculoskeletal soft-tissue injuries. This could explain the finding of a significant linear trend in the *COL5A1 Bst*UI RFLP CC genotype frequency and an increased chronological age in the male subjects analysed. This proposed explanation is further supported by a departure from Hardy-Weinberg equilibrium as observed in some of the groups presented in our previous studies.[3]

It remains unknown why a similar trend was not observed in females. It does however suggest that the *COL5A1 Bst*UI RFLP, as a risk factor for musculoskeletal soft-tissue injuries, is not age dependent in females.

A limitation of this study was that it was not possible to analyse the South African and Australian data separately owing to small sample sizes and uneven genotype distribution. A further limitation was that, although all participants were asymptomatic with regard to a previous history of tendon injuries,[3-5] not all were free of ligament injuries (owing to the study designs).

In conclusion, there was an age-dependent significant increase in distribution of the *COL5A1 Bst*UI RFLP CC genotype in the pooled asymptomatic male participants of the three studies which previously investigated this polymorphism as a possible risk factor for soft-tissue injuries. The practical implication of this finding is that the selection of control groups is of critical importance when future studies of this nature are designed. Future research investigating this genetic variant as a risk factor for soft-tissue injuries should consider the findings of this study when selecting an asymptomatic control group.

Perspective

Genetic variants, such as the *COL5A1 Bst*UI RFLP, may have a significant impact on the prevention of musculoskeletal soft-tissue injures.[6] Genetic variants, together with other intrinsic and extrinsic risk factors, should eventually be used to identify individuals at increased risk of injury. Once individuals are identified as 'at risk', carefully designed intervention programmes should be prescribed to

prevent the injury from occurring and to assist the clinical management of these individuals. The current study provides further information on the *COL5A1 Bst*UI RFLP. The findings may help future studies investigating this genetic variant as a risk factor for musculoskeletal soft-tissue injuries and thereby assist future multifactorial risk models.

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Competing interests. None.

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