The *COL5A1* Gene Is Associated With Increased Risk of Anterior Cruciate Ligament Ruptures in Female Participants

Michael Posthumus,* PhD, Alison V. September,* PhD, Dion O'Cuinneagain,† MBChB, Willem van der Merwe,† MBChB, BMed Sports Hons, FCS(SA), Martin P. Schwellnus,* MBBCh, MSc(Med), MD, FACSM, FFIMS, and Malcolm Collins,*‡§ PhD

*From the *UCT/MRC Research Unit for Exercise Science and Sports Medicine of the Department of Human Biology, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa, the †Sports Science Orthopaedic Clinic, Cape Town, South Africa, and the ‡South African Medical Research Council, Cape Town, South Africa*

Background: Anterior cruciate ligament ruptures, especially to young female athletes, are a cause of major concern in the sports medicine fraternity. The major structural constituents of ligaments are collagens, specifically types I and V. Recently, the gene that encodes for the α1 chain of type I collagen (*COL1A1*) has been shown to be associated with an increased risk of cruciate ligament ruptures. The *COL5A1* gene, which encodes for the α1 chain of type V collagen, has been shown to be associated with Achilles tendon injuries.

Purpose: The study was conducted to determine (1) if 2 sequence variants (*Bst*UI and *Dpn*II restriction fragment length polymorphisms [RFLPs]) within the *COL5A1* gene are associated with an increased risk of anterior cruciate ligament ruptures, and (2) if there were any gender-specific positive associations between the 2 *COL5A1* sequence variants and risk of anterior cruciate ligament ruptures.

Study Design: Case control study; Level of evidence, 3.

Methods: A total of 129 white participants (38 women) with surgically diagnosed anterior cruciate ligament ruptures and 216 physically active control participants (84 women) without any history of ACL injury were included in this case-control genetic association study. All participants were genotyped for the *COL5A1 Bst*UI and *Dpn*II RFLPs.

Results: There was a significant difference in the *Bst*UI RFLP genotype frequency between the anterior cruciate ligament rupture and physically active control groups among the female participants, but not the male participants. The CC genotype in the female participants was significantly underrepresented in the anterior cruciate ligament rupture group compared with the controls (27.4% vs 5.6%; odds ratio = 6.6; 95% confidence interval, 1.5-29.7; $P = .006$). There were no differences in the *DpnII* RFLP genotype distributions between the anterior cruciate ligament rupture and physically active control groups.

Conclusion: The CC genotype of the *COL5A1 Bst*UI RFLP was underrepresented in female participants with anterior cruciate ligament ruptures.

Clinical Relevance: This is the first study to show that there is a specific genetic risk factor associated with risk of anterior cruciate ligament ruptures in female athletes.

Keywords: anterior cruciate ligament (ACL); soft tissue; collagen; tear; polymorphism

Although the exact cause of anterior cruciate ligament (ACL) ruptures is unknown, various intrinsic and extrinsic risk factors have been identified.[4] Intrinsic risk factors include anatomic, hormonal, biomechanical, and neuromuscular factors.[4,5] The major structural components of ligaments are collagens, of which types I and V are the 2 main constituents.[2] The *COL1A1* and *COL5A1* genes encode for the major α chains that make up these collagens, and sequence variants within these genes are potential genetic intrinsic risk factors for ACL ruptures.[17,18] Recently, the rare TT genotype of the functional Sp1 binding site polymorphism within the first intron of the *COL1A1* gene, which encodes for the α1 chain of type I collagen, was shown to be underrepresented in Swedish

§Address correspondence to Malcolm Collins, PhD, UCT/MRC Research Unit for Exercise Science and Sports Medicine, PO Box 115, Newlands, 7725, South Africa (e-mail: malcolm.collins@uct.ac.za).

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patients with cruciate ligament ruptures and shoulder dislocations.[7] Similarly, in another study, we have shown that this genotype is also underrepresented in South African patients with ACL ruptures.[13] Because ACL ruptures are complex conditions, it is unlikely that this is the only genetic variant associated with risk of ACL ruptures.[18] Sequence variants within several genes, including *COL5A1*,[11,16] *TNC* (tenascin C),[10] and *MMP3* (matrix metalloproteinase 3),[14] have to date been shown to be associated with Achilles tendinopathy, another common soft tissue injury that occurs during sports participation.

The *COL5A1* gene encodes for the α1 chain of type V collagen, which is a minor fibrillar collagen found in ligaments and tendons, as well as other tissues.[6] Type V collagen, which makes up approximately 10% of the collagen content in ligaments,[12] intercalates into the core of type I collagen fibrils, where it is believed to be involved in the organization and regulation of type I collagen fibril diameters.[12] The CC genotype of the *Bst*UI restriction fragment length polymorphism (RFLP) within the 3′-untranslated region (UTR) of the *COL5A1* gene has been shown to be significantly underrepresented in participants with chronic Achilles tendinopathy.[11,16] Because tendons and ligaments have a similar composition and hierarchical structure,[2,19] the *COL5A1* gene is an ideal candidate gene for investigation as a possible additional genetic risk factor for ACL ruptures.

The primary aim of this study was therefore to determine if 2 sequence variants (the *Bst*UI and *Dpn*II RFLPs) within the 3′-UTR of the *COL5A1* gene are associated with an increased risk of ACL ruptures. Although a relatively larger number of ACL ruptures occur in men, women have up to 4.6 times greater risk of ACL rupture than men.[9] Specific intrinsic risk factors have been implicated in the increased susceptibility of ACL ruptures in women; however, the cause of the increased risk is still unknown. A secondary aim of this study was therefore to determine if there were any gender-specific associations between the 2 *COL5A1* sequence variants and increased risk of ACL ruptures.

MATERIALS AND METHODS

Participants

A total of 129 white participants (38 women and 91 men) with surgically diagnosed ACL ruptures were recruited for this study from the Sports Science Orthopaedic and Sports Medicine Clinics in Cape Town, South Africa. In addition, 216 apparently healthy, unrelated, physically active, white participants (84 women and 132 men), without any self-reported history of ACL injury, were recruited as control (CON) participants from sports and recreational clubs within the greater Cape Town area of South Africa. Control participants were physically active, participated in similar sports, and were within a similar age category as the ACL ruptures group.

Before participation in this study, all the participants gave informed written consent. In addition, each participant completed personal details, medical history, personal and blood relative (family) ligament and tendon injury history, as well as a sports participation questionnaire. Sports participation was categorized into contact sports, noncontact jumping sports, noncontact nonjumping sports, and skiing sports, as previously defined,[1] with slight modification. Contact sports included soccer, rugby, touch rugby, Gaelic football, muay thai, hurling, Australian football league, and boxing. Noncontact jumping sports included netball, basketball, volleyball, gymnastics, ballet, motocross, skateboarding, paragliding, handball, and skydiving. Noncontact nonjumping sports included field hockey, cricket, tennis, horseback riding, running, bicycling, spinning, squash, swimming, aerobics, yachting, athletics (excluding long and triple jump), golf, dancing, tennis, canoeing, water polo, surfing, windsurfing, badminton, gym training, bowls, triathlon, softball, and lifesaving. Skiing sports included any mode of water or snow skiing.

The exact mechanism of injury could only be identified in 21 female (55.7%) and 67 male (73.6%) participants. Participants who had ruptured their ACL via a noncontact (NON) mechanism, as previously defined,[9] were identified and analyzed in this study as a separate subgroup. Thirty-six male participants (53.7%) and 18 female participants (85.7%) ruptured their ACL through a noncontact mechanism.

This study was approved by the Research Ethics Committee of the Faculty of Health Sciences within the University of Cape Town, South Africa (reference number 164/2006).

DNA Extraction and *COL5A1* Genotyping

Approximately 4.5 mL of venous blood was obtained from each participant by venipuncture of a forearm vein and collected into an ethylenediaminetetraacetic acid vacutainer tube. Blood samples were stored at 4°C until total deoxyribonucleic acid (DNA) extraction. The DNA was extracted using the procedure described by Lahiri and Nurnberger[8] and modified by Mokone et al.[10] A 667-bp fragment containing the *Dpn*II (single nucleotide polymorphism [SNP] rs13946) and *Bst*UI (SNP rs12722) RFLPs within the 3′-UTR of the *COL5A1* gene were amplified with polymerase chain reaction as described by Greenspan and Pasquinelli[3] and modified by Mokone et al.[11] The C and T alleles (alternative forms of a specific gene) of the 2 polymorphisms (sequence variants) were identified by digesting the polymerase chain reaction products with the restriction enzymes *Bst*UI or *Dpn*II, as previously described.[11,16] The resultant fragments were separated, together with a 100-bp DNA ladder of known size markers (Promega Corporation, Madison, Wisconsin) and SYBR Gold nucleic acid gel stain (Invitrogen Molecular Probes, Eugene, Oregon), on 6% nondenaturing polyacrylamide gels. The gels were photographed under ultraviolet light using a Uvitec photodocumentation system (Uvitec Limited, Cambridge, United Kingdom) and genotypes were determined based on the sizes of the DNA fragments.

TABLE 1
Characteristics of the Female and Male Participants Within the Asymptomatic Control (CON) Group,the Anterior Cruciate Ligament Rupture Group (ACL), and the ACL Subgroup With a Noncontact Mechanism of Injury (NON)[a]

	CON	ACL	*P* Value[b]	NON	*P* Value[c]
Female participants (no.)	84	38		18	
Age (y)	28.2 ± 10.0 (83)	29.8 ± 12.1 (37)	.453	28.6 ± 13.1 (18)	.896
Height (cm)	166.2 ± 5.8 (210)	166.4 ± 6.8 (34)	.929	167.8 ± 6.7 (17)	.325
Weight (kg)	61.9 ± 8.3 (82)	62.2 ± 7.5 (34)	.864	63.2 ± 7.8 (17)	.570
BMI (kg/m^2)	22.4 ± 2.7 (79)	22.3 ± 2.1 (33)	.940	22.1 ± 2.0 (16)	.727
Country of birth (% South African)	82.5 (80)	80.0 (35)	.955	77.8 (18)	.737
Male participants (no.)	132	91		36	
Age (y)	29.0 ± 12.2 (132)	28.1 ± 10.5 (87)	.584	27.6 ± 9.7 (36)	.512
Height (cm)	180.4 ± 6.3 (131)	181.1 ± 6.6 (79)	.450	181.8 ± 6.6 (35)	.436
Weight (kg)	82.0 ± 13.9 (130)	87.3 ± 14.3 (80)	**.008**	87.5 ± 14.7 (36)	**.040**
BMI (kg/m^2)	25.1 ± 3.8 (129)	26.6 ± 3.7 (76)	**.010**	26.7 ± 4.2 (34)	**.037**
Country of birth (% South African)	89.0 (127)	85.4 (82)	.577	88.9 (36)	1.000

[a]Gender and country of birth are represented as a frequency (%). The remaining variables are expressed as a mean ± standard deviation. The number of participants (n) for each variable is in parentheses.

Age, weight and body mass index (BMI) are self-reported values at the time of the first ACL rupture for the ACL group, as well as the NON subgroup, and at recruitment for the CON group. For the ACL group, age, weight, and BMI at recruitment were 6.4 ± 9.9 years (n = 37), 0.7 ± 4.5 kg (n = 34), and 0.4 ± 1.5 kg/m^2 (n = 33) greater than at the time of the first ACL rupture for the female participants; and 4.3 ± 7.7 years (n = 83), 1.6 ± 4.7 kg (n = 76), and 0.5 ± 1.5 kg/m^2 (n = 73) greater than at the time of the first ACL rupture for the male participants.

[b]CON vs ACL. **Boldface type** indicates significance ($P < .05$).

[c]CON vs NON. **Boldface type** indicates significance ($P < .05$).

Statistics

Data were analyzed using Statistica Version 8.0 (Statsoft Inc., Tulsa, Oklahoma) and Graphpad InStat Version 3 (Graphpad Software, San Diego, California) statistical programs. The data were initially analyzed for the whole group and then separately according to genders. Because the sample was predominantly men, the whole group results were similar to the men. Only the separate male and female results are reported in this study. A 1-way analysis of variance was used to determine any significant difference between the characteristics of the ACL and CON groups, as well as the CON group and NON subgroup. A χ^2 analysis or Fisher exact test was used to analyze any differences in the genotype and allele frequencies, as well as other categorical data between the groups. Significance was accepted when $P < .05$. Hardy-Weinberg equilibrium was established using the program Genepop Web version 3.4 (http://genepop.curtin.edu.au/).

RESULTS

Participant Characteristics

There were no significant differences in the proportion of female participants within the CON (n = 84, 38.9%) and ACL (n = 38, 29.5%) groups ($P = .260$), as well as between the CON group (n = 84, 38.9%) and NON subgroup (n = 18, 33.3%; $P = .715$) (Table 1). The female and male participants within the CON and ACL groups, as well as the NON subgroup, were matched for age, height, and country of birth (Table 1). The female CON and ACL groups, as well as the NON subgroup, were also matched for weight and body mass index (BMI). The male participants within the ACL group and NON subgroup were, however, significantly heavier and had a significantly higher BMI than the CON group. Within the ACL group, the female participants' self-reported age, weight, and BMI were greater at recruitment than at the time of the first ACL rupture *by* 6.4 ± 9.9 years (n = 37), 0.7 ± 4.5 kg (n = 34), and 0.4 ± 1.5 kg/m^2 (n = 33), respectively. The male participants' self-reported age, weight, and BMI were greater at recruitment than at the time of the first ACL rupture *by* 4.3 ± 7.7 years (n = 83), 1.6 ± 4.7 kg (n = 76), and 0.5 ± 1.5 kg/m^2 (n = 73), respectively.

The relative frequency of the self-reported history of any other (excluding ACL) ligament ($P = .003$) and other knee ligament ($P = .029$) injuries were significantly higher in the female ACL group when compared with the female CON group (Table 2). The previous other knee ligament injuries included injury to either the posterior cruciate ligament, lateral collateral ligament, or medial collateral ligament. Similarly, the female participants within the NON subgroup also reported significantly greater history of any other previous ligament ($P = .014$) and knee ligament ($P = .018$) injuries compared with the CON group. It is interesting to note that the self-reported family history of any ligament injury at the time of recruitment was significantly higher in the female ACL group ($P = .002$), as well as the NON subgroup ($P = .014$), when compared with the female CON group. Except for a significant difference in the frequency of other knee ligament injuries ($P = .047$) between the male ACL and CON groups, there were no significant differences between the ACL and CON groups,

TABLE 2
Self-Reported Personal and Family (Blood Relative) History of Soft Tissue Injuries Within the Asymptomatic Control (CON) group, the Anterior Cruciate Ligament Rupture Group (ACL), and the ACL Subgroup With a Noncontact Mechanism of Injury (NON) in the Female and Male Participants[a]

	CON	ACL	*P* Value[d]	NON	*P* Value[e]
Female participants (no.)	84	38		18	
Any other ligament injury[b]	25.9 (81)	54.3 (35)	**.003**	55.6 (18)	**.014**
Knee ligament injury[c]	1.2 (81)	11.4 (35)	**.029**	16.7 (18)	**.018**
Achilles tendon injury	4.9 (81)	5.7 (35)	1.000	11.1 (18)	.299
Family ligament injury	21.5 (79)	50.0 (34)	**.002**	52.9 (17)	**.014**
Male participants (no.)	132	91		36	
Any other ligament injury[b]	43.6 (124)	57.5 (80)	.051	54.3 (35)	.260
Knee ligament injury[c]	4.8 (124)	12.5 (80)	**.047**	8.6 (35)	.414
Achilles tendon injury	10.5 (124)	11.1 (81)	.887	20.0 (35)	.134
Family ligament injury	27.1 (122)	33.8 (80)	.308	34.3 (35)	.404

[a]Values are represented as frequencies (%) with the number of participants (n) in parentheses.
[b]Excludes ACL injuries.
[c]Includes the posterior cruciate ligament, the lateral collateral ligament, and the medial collateral ligament.
[d]CON vs ACL. **Boldface type** indicates significance ($P < .05$).
[e]CON vs NON. **Boldface type** indicates significance ($P < .05$).

as well as between the CON group and NON subgroup, for a history of Achilles tendon injury or any other category of personal or family history of ligament injury. The sports participation details of the female and male CON and ACL groups are summarized in the Appendix (see online Appendix for this article at http://ajs.sagepub.com/supplemental/).

COL5A1 Genotype and Allele Frequencies

When the female and male participants were analyzed together, there were no significant differences in genotype or allele frequencies between the CON and ACL groups, nor the CON group and NON subgroup for the *COL5A1* *Bst*UI and *Dpn*II genotypes (data not shown). However, when the female and male participants were analyzed separately, the *Bst*UI RFLP CC genotype was overrepresented in the control participants within the female (Figure 1A) (odds ratio [OR] = 6.6; 95% confidence interval [CI], 1.5-29.7; $P = .006$), but not the male (Figure 1B; $P = .987$) participants, when the ACL groups were compared with the CON groups. The allele frequencies between the CON and ACL groups for the *Bst*UI genotype was also significantly different within the female participants (OR = 2.2; 95% CI, 1.2-4.0; $P = .010$), with the T allele being overrepresented in the ACL group, but not male participants ($P = .960$; data not shown). Similarly, the T allele of the *Bst*UI RFLP was also significantly overrepresented in the female NON subgroup (OR = 2.6; 95% CI, 1.2-5.7; $P = .016$) when compared with the CON group (data not shown). These data should be interpreted with caution because of the small sample size; however, the genotype frequency of the *COL5A1* *Bst*UI was not significantly different when the female CON group and NON subgroup were analyzed (Figure 1A), although a trend existed for the CC to be overrepresented in the control population ($P = .065$). There were no further significant *Bst*UI genotype ($P = .879$; Figure 1B) or allele ($P = .789$; data not shown) frequency differences when the male CON group and NON subgroup were analyzed.

There were also no significant differences in the *COL5A1* *Dpn*II RFLP genotype or allele frequency distributions between the female (Figure 1C and data not shown) or male (Figure 1D and data not shown) CON and ACL groups, as well as the NON subgroup.

Similar *Bst*UI genotype distributions were obtained when the subgroup of 48 female (31.2% TT, 43.8% TC, and 25.0% CC) and 57 male (29.8% TT, 56.1% TC, and 14.04% CC) control participants without a self-reported history of any ligament or tendon injuries were analyzed separately. The genotype distribution of the female and male CON (*Bst*UI, $P \geq .283$; *Dpn*II, $P \geq .429$), as well as the female and male ACL (*Bst*UI, $P \geq .391$; *Dpn*II, $P \geq .132$) groups, were in Hardy-Weinberg equilibrium for the *Bst*UI and *Dpn*II RFLPs.

Lastly, it is interesting to note that there was a significant difference in the *Bst*UI genotype distribution when all participants (ACL and CON) in the study were divided into those with and without a family history of ligament injuries ($P = .022$). This association was also significant when only the female (Figure 2A; TT vs TC + CC, $P = .005$), but not the male (Figure 2B; $P = .396$), participants were analyzed. In the female participants, the TT genotype was significantly overrepresented (OR = 3.6; 95% CI, 1.5-8.3; $P = .005$) in those with a family history of ligament injury (n = 34; TT, 52.9%) when compared with those without a family history of ligament injury (n = 79; TT, 24.1%). The CC genotype was, however, not significantly underrepresented ($P = .203$) in those with a family history of ligament injury (n = 34; CC, 11.8%) when compared with those without a previous family history of ligament injury (n = 79; CC, 24.1%).

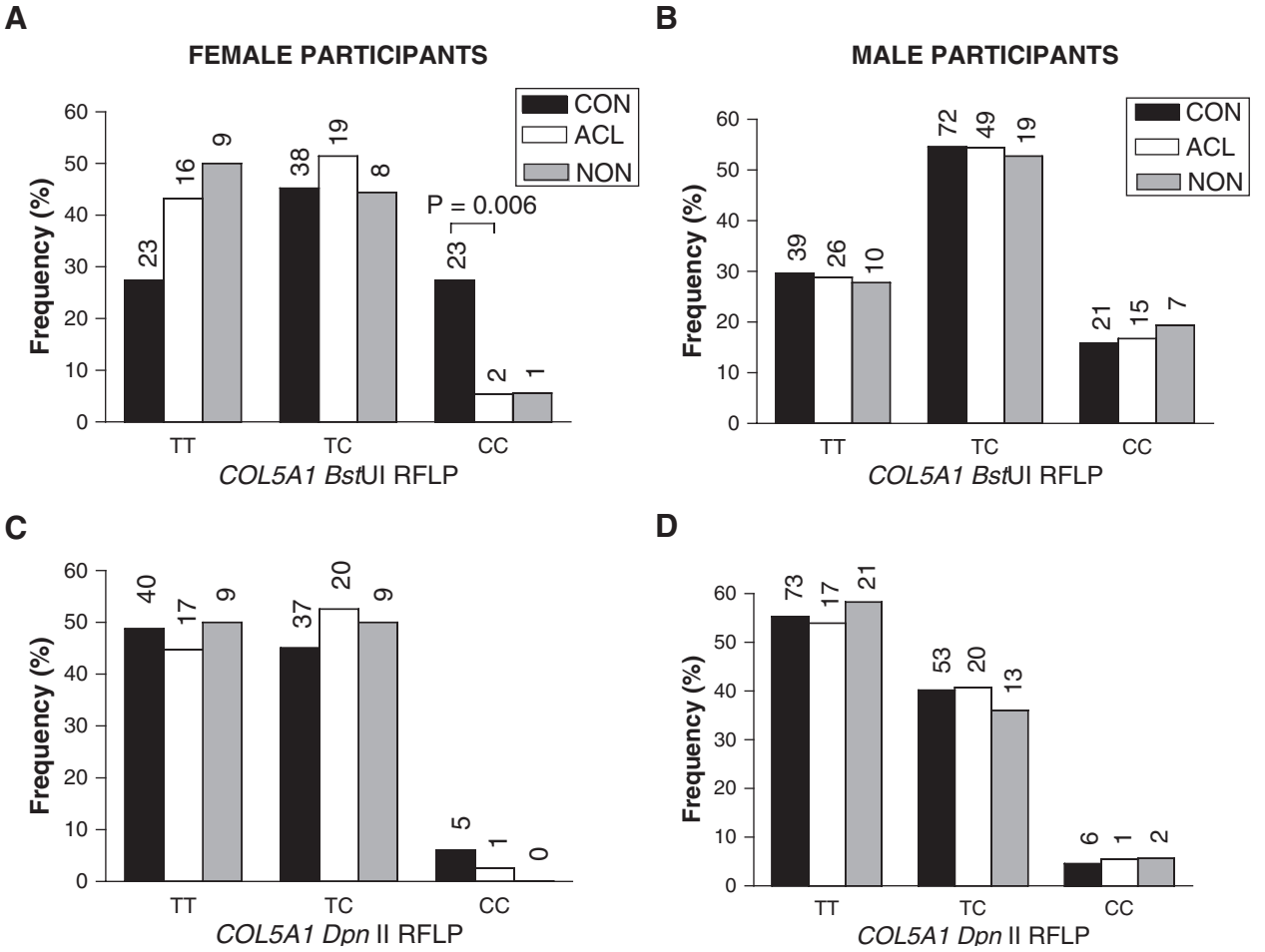

Figure 1. The relative genotype frequency of the *COL5A1* gene *Bst*UI and *Dpn*II restriction fragment length polymorphisms (RFLPs) within the asymptomatic control (CON; black bars) group, the anterior cruciate ligament rupture group (ACL; white bars), and the noncontact mechanism of injury subgroup (NON; gray bars) in all female (A and C) and male (B and D) participants. Because of the small sample size of the *Bst*UI RFLP CC genotype in the female ACL and NON groups (A), the CC genotype was compared with the combined TC and TT genotypes. The TT genotype was also compared with the combined TC and CC genotypes. Similarly, because of the small sample size of the rare *Dpn*II RFLP CC genotype (C and D), it was combined with the TC genotype and compared with the TT genotype. A, the *Bst*UI RFLP genotype distributions within the female participants. CON vs ACL: $P = .006$ (CC vs TT + TC) and $P = .131$ (TT vs TC + CC); CON vs NON: $P = .065$ (CC vs TT + TC) and $P = .110$ (TT vs TC + CC). B, the *Bst*UI RFLP genotype distributions within the male participants. CON vs ACL, $P = .987$; CON vs NON, $P = .879$. C, the *Dpn*II RFLP genotype distributions within the female participants. CON vs ACL, $P = .829$ (TT vs TC + CC); CON vs NON, $P = .925$ (TT vs TC + CC). D, the *Dpn*II RFLP genotype distributions within the male participants. CON vs ACL, $P = .938$ (TT vs TC + CC); CON vs NON, $P = .892$ (TT vs TC + CC). The number of participants (n) in each genotype group is indicated above each bar.

DISCUSSION

The main finding of this study was that the CC genotype of the 1 variant (the *Bst*UI RFLP) within the 3′-UTR of the *COL5A1* gene was underrepresented in female (OR = 6.6; 95% CI, 1.5-29.7; $P = .006$), but not male, participants with ACL ruptures. The second variant (the *Dpn*II RFLP) was not associated with ACL ruptures in either the female or male participants. A second finding of this study was that the female, but not male, participants within the ACL group reported a significantly higher family history of ligament injuries. The *Bst*UI genotype was also associated with a family history of ligament injuries in the female participants.

Our novel finding, that female participants with a CC genotype of the *COL5A1 Bst*UI RFLP had a decreased risk of ACL ruptures, has not been previously reported. The *COL5A1* gene encodes for the α1 chain in type V collagen, which is an important structural constituent of both ligaments and tendons.[6] It is therefore of interest to note that we have previously shown that this same CC genotype was also associated with a decreased risk of chronic Achilles tendinopathy in men and women.[11,16] Similarly, the second variant, the *DpnII* RFLP, of the *COL5A1* gene was not

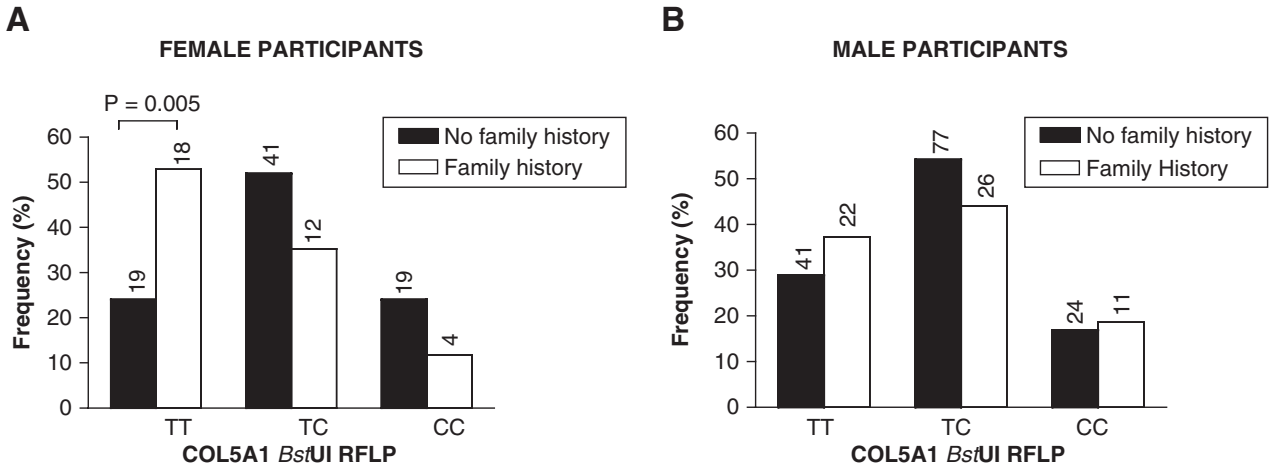

Figure 2. The relative genotype frequency of the *COL5A1* gene *Bst*UI and *Dpn*II restriction fragment length polymorphisms (RFLPs) when female (A) and male (B) participants in the study were divided into those with and without a family history of ligament injuries. A, the *Bst*UI RFLP genotype distributions within the female participants: TT vs TC + CC, $P = .005$; CC vs TC + TT, $P = .203$. B, the *Bst*UI RFLP genotype distributions within the male participants; $P = .396$. The number of participants (n) in each genotype group is indicated above each bar.

associated with either ACL ruptures in this study or chronic Achilles tendinopathy from previous studies.[11,16] Also of importance is that our finding did not change when participants in this study who reported a history of Achilles tendon injury were excluded from the analysis (data not shown).

Our second finding, that the female participants within the ACL group reported a significantly higher family history of ligament injuries, is also supported by data from 1 other study.[1] In that study, siblings of individuals who had ruptured an ACL were twice as likely to rupture their ACL.[1] Although it is possible that the family members of the ACL participants in our study were also exposed to a greater amount of contact sports, or other extrinsic risk factors for ligament injuries, this observation is consistent with a familial predisposition to an increased risk of ligament injuries. In support of this familial predisposition, our study showed that the *Bst*UI RFLP within *COL5A1* was associated with a self-reported family history of ligament injuries in the female, but not male, participants. However, it is not clear why this observation was only present in female participants.

Although various intrinsic risk factors have been proposed to be associated with female ACL ruptures,[5,15] the exact mechanism by which these factors contribute to an increased risk of ACL ruptures in women remain unknown. Certain intrinsic risk factors broadly classified as either hormonal, anatomic, neuromuscular, or biomechanical, might be specific or exaggerated in women.[5] One hypothesis may be that gene-hormone interactions exist, which makes this genetic association specific to women. Sex hormones are known to exert their biologic effects on the ACL through regulating gene expression, especially many of the MMPs.[14] Although we are not aware of previous research investigating the effect of female sex hormones on the regulation of *COL5A1* gene expression, previous research has shown that relative to *COL1A1* gene expression, *MMP3* and *MMP1* gene expression is higher in the ACL of women when compared with men.[20] It is therefore interesting to note that Raleigh et al[14] recently reported an interaction between a sequence variant within the *MMP3* gene and the *COL5A1 Bst*UI RFLP that modifies the risk of Achilles tendinopathy. Further studies are required to determine whether variants within the *MMP3* gene are associated with ACL ruptures, particularly within women.

Because ACL ruptures are complex disorders, some nongenetic factors that are potential confounding variables in injury risk need to be discussed. In our study, the female participants within the CON and ACL groups were matched for age, height, body weight, BMI, and country of birth. It has previously been shown that body weight and BMI are risk factors for ACL ruptures in women, but not men.[21] Female participants were also matched for participation in contact sports, noncontact nonjumping sports, and skiing sports. Significantly more women within the ACL group participated in noncontact jumping sports when compared with the control group. As participation in noncontact jumping sports increases the risk of ACL ruptures, this is a potential limitation of this study. There were, however, no genotype effects with sports participation in this study (data not shown). It is important to note that significantly more noncontact ACL ruptures were observed in the female group (85.7%) when compared with the male group (53.7%). This may be expected, as it is widely reported that women are at a greater risk than men to develop noncontact ACL ruptures.

Among the male participants in the current study, the ACL group and NON subgroup were significantly heavier, and had a significantly higher BMI than the CON group. Although the possibility cannot be excluded, weight and BMI are not documented risk factors for male ACL ruptures.[21] The ACL group had, however, reported playing a significantly greater amount of contact sports. The fact that our male controls were not matched for participation

in contact sports is a limitation of our study. It must however be noted that weight, BMI, or exposure to contact sports had no effect on genotype distributions. Furthermore, no genotype associations were observed when a subgroup of the male participants were matched according to weight and exposure to contact sports (data not shown).

Another limitation of our study was the relatively small sample size of the female participants. The primary aim of our study was not intended to investigate gender-specific genetic risk factors, and therefore further research is required to confirm this finding in larger female cohorts.

In conclusion, the CC genotype of the *Bst*UI RFLP with the 3′-UTR of the *COL5A1* gene is associated with reduced risk of ACL ruptures in female participants in this cohort. This work needs to be repeated in independent populations. Further well-controlled prospective studies are eventually required to confirm these findings and to accurately assess the risk of ACL ruptures based on this variable.

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