

## Collagen Gene Variants Previously Associated With Anterior Cruciate Ligament Injury Risk Are Also Associated With Joint Laxity

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### **Abstract:**

**Background:** Genetic association studies demonstrate a relationship between several collagen gene variants and anterior cruciate ligament (ACL) injury, yet the mechanism of these relationships is still unclear. Joint laxity is a heritable trait; increased magnitudes of anterior knee laxity (AKL), genu recurvatum (GR), and general joint laxity (GJL) have been consistently associated with a greater risk of ACL injury. Joint laxity may constitute an important intermediate phenotype for the genetic association with ACL injury that can be measured clinically.

**Hypothesis:** To determine if genetic variants within the *COL1A1*, *COL5A1*, and *COL12A1* genes, previously associated with ACL injury, were also associated with greater magnitudes of AKL, GR, and GJL.

**Study Design:** Descriptive laboratory study.

**Methods:** Blood samples and measures of AKL, GR, and GJL were obtained from 124 (50 male, 74 female) healthy, recreationally active subjects. Genomic DNA was extracted from the blood samples and genotyped for single-nucleotide polymorphisms previously examined relative to ACL injury. Univariate analyses of variance compared the magnitude of each laxity variable across the 3 genotypes for each single-nucleotide polymorphism in both sex-combined and sex-specific models.

**Results:** Specific genotypes were associated with greater GR in all subjects. Some genotypes were associated with greater magnitudes of GR, AKL, and GJL in females only.

**Conclusions:** Gene variants previously associated with ACL injury risk were in large part also associated with joint laxity. Sex-specific genetic associations with joint laxity were consistent with those previously reported for ACL injury.

**Clinical Relevance:** These data provide insight into potential pathways through which genotypic variants in collagen genes have the potential to alter ligament structure and behavior and, thus, ACL injury risk.

**Keywords:** joint laxity | genetic variations | anterior cruciate ligament injury | collagen genes | sex differences

### Article:

Rupture of the anterior cruciate ligament (ACL) is a debilitating injury that is costly both in time loss from sport and in money spent on surgery and rehabilitation.<sup>14</sup> The heightened risk of subsequent ligament injury<sup>36,41,47</sup> and the development of osteoarthritis within 10 to 15 years after the initial injury are of particular concern.<sup>22</sup> Over 70% of ACL injuries are noncontact in nature,<sup>4</sup> and females more often experience noncontact injuries than similarly trained males.<sup>25</sup> While a familial predisposition for ACL injury risk is known,<sup>10,16,29,31</sup> only recently have specific genetic changes (single-nucleotide polymorphisms [SNPs]) within the genes coding for collagen types I, V, and XII (*COL1A1*, *COL5A1*, and *COL12A1*) been associated with ACL injury, particularly in females.<sup>18,29-31</sup> Despite these associations, the mechanisms tying these genetic variants to ACL injury risk have not been elucidated.

The effect of collagen genes and the variations within them may play a critical role in explaining ACL injury risk given the structural importance of collagen within ligaments. Type I collagen is present in the ACL<sup>9</sup> and is the major fibrillar collagen providing structural stability to ligaments.<sup>5</sup> Type V is also known to be fibrillar in structure (similar to that of type I)<sup>5</sup> and to intercalate with type I to affect fibrillogenesis.<sup>3</sup> Type XII is a fibril-associated collagen with interrupted triple helices, and, as the name suggests, it is known to interact on the surface of fibrillar collagens and potentially provides a structural role.<sup>48</sup> Since collagen is the major component of ligaments (75% by dry weight),<sup>11</sup> these findings suggest that these collagen gene variants have the potential to alter the structural integrity of the ligament, which may be manifested through changes in joint laxity (a measure that in large part reflects the behavior of ligament structures to an externally applied load). While research has yet to examine joint laxity as a potential intermediate phenotype for these genetic associations with ACL injury, both epidemiology and familial association studies suggest that this potential intermediate role is plausible.

Joint laxity is a highly heritable trait,<sup>2,6,8,15,40</sup> and increased magnitudes of joint laxity have consistently been implicated as an ACL injury risk factor.<sup>19,23,26,32,34,43,46</sup> Specifically, studies report a familial predisposition to greater joint laxity or joint laxity pathologies (ie, shoulder dislocation),<sup>2,6,8,40</sup> and a greater prevalence of general joint laxity (GJL) in female identical twins

compared with fraternal twins<sup>15</sup> supports this heritability. In a prospective injury risk study measuring joint laxity among other variables in 859 military cadets over a 4-year period reported that individuals with greater GJL (> 5 out of 9) and anterior knee laxity (AKL) (1 standard deviation above the mean) had a 2.8- and 2.6-fold greater risk, respectively, of rupturing their ACL.<sup>43</sup> When parsed by sex, females who had high AKL remained at a 2.7-fold greater risk, but this risk was not identified in males.<sup>43</sup> Another prospective injury risk study measuring knee hyperextension (herein, genu recurvatum [GR]) and AKL reported that greater magnitudes of GR and side-to-side differences in AKL contributed significantly to a multivariate logistic regression model for ACL injury risk.<sup>26</sup> Numerous retrospective studies have consistently associated greater magnitudes of GJL,<sup>19,23</sup> AKL,<sup>34,46</sup> and GR<sup>19,32</sup> with ACL injury risk.

Based on these findings, characterizing the relationship between joint laxity and the collagen gene variants previously associated with ACL injury may help elucidate potential mechanisms tying genetic variants to ACL injury risk. Therefore, the purpose of this candidate gene association study was to determine if genetic variants within the *COL1A1*, *COL5A1*, and *COL12A1* genes previously associated with ACL injury<sup>8,29-31</sup> were also associated with greater magnitudes of joint laxity (AKL, GR, and GJL). Our expectation was that collagen gene variants that have been previously associated with ACL injury, given their potential to alter ligament structure, would also be associated with greater magnitudes of joint laxity.

## Methods

Data for this study were obtained from 124 subjects (50 male: 22.2 ± 2.8 years, 177.9 ± 9.3 cm, 80.9 ± 13.3 kg; 74 female: 21.4 ± 2.6 years, 163.9 ± 6.7 cm, 61.1 ± 8.8 kg), from whom we had previously obtained blood samples and knee laxity data.<sup>37,39</sup> Participants were recreationally active between 2.5 and 10 hours per week, had a body mass index < 30, and had no history of connective tissue disorders or knee injury. Female participants had normal menstrual cycles and had not used hormone-based medications for the past 6 months. Participants signed a consent form approved by the university institutional review board prior to enrollment.

Because knee laxity can vary across the menstrual cycle in a nonuniform fashion among women,<sup>28,37</sup> we took repeated measurements across 2 cycles to obtain each individual's minimum (baseline) laxity values.<sup>37</sup> AKL was measured in millimeters as the anterior displacement of the tibia relative to the femur when a 133-N posterior-to-anterior directed load was applied to the tibia using the KT-2000 Knee Arthrometer (MEDmetric Corp, San Diego, California).<sup>37</sup> GR was measured in degrees with a standard goniometer as the amount of knee hyperextension (positive value) when the subject maximally contracted his or her quadriceps and extended the knee with the distal shank on a 4-in. bolster.<sup>37</sup> GJL was measured with the Beighton and Horan Joint Mobility Index,<sup>1</sup> which measures the presence of joint hypermobility at 5 anatomic locations (thumb, wrist, elbow, and knee bilaterally and forward trunk flexion), scoring 1 point for hypermobility at each joint. Measurement reliability values for these laxities were

confirmed prior to testing and found to be 0.97 (0.4 mm) for AKL, 0.97 (0.5°) for GR, and 0.98 (0.3 points) for GJL.<sup>37</sup>

Blood samples (10 mL) were collected from the subject's antecubital vein during the morning hours and stored at -80°C. DNA was isolated with the DNAeasy Extraction Kit (Qiagen, Valencia, California), and genotyping of the target SNPs was conducted by GeneSeek (Lincoln, Nebraska). Prior to genotyping, a random subset of samples were subjected to standard polymerase chain reaction and electrophoresis to verify the presence of genomic DNA. SNPs were genotyped, including all of those previously investigated for ACL injury,<sup>18,29-31</sup> whether or not significant associations were reported (Table 1). By including SNPs not previously associated with ACL injury, our goal was to minimize the possibility that there were nonspecific genetic associations with knee laxity and to further strengthen the hypothesis that any genetic associations with joint laxity produced from this study would be consistent with genetic associations previously reported for ACL injury.

**Table 1.** Location and effects of candidate single-nucleotide polymorphisms.

Polymorphism	Location	Effect
rs1800012	Promoter region, Sp1 binding site of COL1A1	Influences transcription levels of $\alpha 1(I)$ chain of type I collagen <sup>24</sup>
rs12722 rs13946	3' UTR of COL5A1	Regulates mRNA stability <sup>20</sup>
rs240736 rs970547	Coding region of COL12A1	Missense, isoleucine → threonine <sup>27</sup> Missense, glycine → serine <sup>27</sup> Within C-terminal NC1 binding domain <sup>42</sup>

Statistical analysis was conducted with PASW Statistics 18 (IBM SPSS Statistics, New York, New York). Race datum was self-reported, and a  $\chi^2$  analysis was used to determine effect on population stratification. Univariate, one-way analyses of variance compared mean AKL, GR, and GJL values among the 3 genotypes for each SNP. Significant main effects were followed by post hoc, pairwise comparisons. Given sex-specific genetic associations with ACL injury previously reported for *COL5A1* and *COL12A1* polymorphisms,<sup>30,31</sup> both sex-combined and sex-specific analyses were performed. Alpha level was set a priori at  $P < 0.05$ . Genotypic variants at each gene locus were tested to determine if they were in Hardy-Weinberg equilibrium (HWE) based on a  $\chi^2$  goodness-of-fit test.<sup>33</sup> A sample in HWE suggests that no genotypes have been disproportionately represented because of a potential selection bias or distortions caused by genetic heterogeneity.

## Results

Genotypic distribution and racial makeup of our sample for the candidate SNPs are reported in Table 2. Of the 124 samples analyzed, the success rate varied from 79.8% to 89.5% for each SNP reaction in this study. HWE results and  $\chi^2$  analysis of race and genotype are also reported in Table 2. All laxity scores are reported as means and standard deviations.

**Table 2.** Population<sup>a,27</sup> and sample genotypic distributions of candidate single-nucleotide polymorphisms.

Polymorphism: Genotype	Sample Frequency, % (No.)				Racial Makeup, % (No.)			
	Population,%	All	Females	Males	Black	Asian	Amer Ind/ Alaska Nat	White
rs1800012								
TT	N/A	5 (5) <sup>b</sup>	5 (3) <sup>b</sup>	4 (2)	0 (0)	0 (0)	0 (0)	6 (5) <sup>b</sup>
TG	N/A	21 (22) <sup>b</sup>	16 (10) <sup>b</sup>	26 (12)	25 (4)	9 (1)	0 (0)	22 (17) <sup>b</sup>
GG	N/A	74 (80) <sup>b</sup>	79 (48) <sup>b</sup>	70 (32)	75 (12)	91 (10)	100 (1)	72 (57) <sup>b</sup>
rs12722								
CC	11b	40 (39) <sup>b</sup>	49 (29) <sup>b</sup>	25 (10)	81 (13) <sup>c</sup>	44 (4)	0 (0)	30 (22) <sup>b</sup>
CT	64b	34 (34) <sup>b</sup>	29 (17) <sup>b</sup>	43 (17)	19 (3)	44 (4)	0 (0)	37 (27) <sup>b</sup>
TT	25b	26 (26) <sup>b</sup>	22 (13) <sup>b</sup>	32 (13)	0 (0) <sup>c</sup>	12 (1)	100 (1)	32 (24) <sup>b</sup>
rs13946								
CC	4	8 (8)	10 (6)	5 (2)	13 (2)	25 (1)	0 (0)	7 (5)
CT	38	33 (35)	30 (18)	39 (18)	19 (3)	20 (7)	0 (0)	32 (25)
TT	58	59 (62)	60 (37)	56 (37)	68 (11)	27 (3)	100 (1)	61 (47)
rs240736								
CC	5	9 (10)	13 (8)	4 (2)	12 (2)	0 (0)	100 (1) <sup>c</sup>	8 (7)
CT	48	38 (42)	39 (25)	37 (17)	50 (8)	27 (3)	0 (0)	39 (31)
TT	47	53 (58)	48 (31)	59 (27)	38 (6)	72 (8)	0 (0)	53 (44)
rs970547								
AA	63	53 (57)	49 (31)	58 (26)	50 (9)	36 (4)	0 (0)	53 (44)
AG	27	43 (46)	44 (28)	40 (18)	44 (8)	55 (6)	100 (1)	43 (31)
GG	10	5 (5)	6 (4)	2 (4)	7 (1)	9 (1)	0 (0)	4 (3)

<sup>a</sup>Frequency of HapMap-CEU. <sup>b</sup>Sample not in Hardy-Weinberg equilibrium. <sup>c</sup>Indicates a significant over- or underrepresentation within the cell ( $\chi^2$ ,  $P \leq 0.05$ ).

The SNP within *COL1A1* (rs1800012) was associated with GR in all subjects ( $P = 0.05$ ) but not in sex-stratified models (females,  $P = 0.17$ ; males,  $P = 0.10$ ). GR was higher in individuals with the TG genotype ( $5.0^\circ \pm 3.1^\circ$ ) compared to the GG genotype ( $2.9^\circ \pm 3.6^\circ$ ;  $P = 0.02$ ). When considering the genotypes that carry the T allele (TT + GT), the association remained significant (TT + GT:  $4.8^\circ \pm 3.3^\circ$  vs GG:  $2.9^\circ \pm 3.6^\circ$ ;  $P = 0.02$ ); that is, the presence of the T variant was

associated with greater GR in both sexes. The male subset was in HWE, while the female subset was not, and no racial bias was indicated for this SNP.

Of the 2 SNPs analyzed within *COL5A1*, a significant association was observed between rs12722 and GJL in all subjects ( $P = 0.02$ ), while there were no associations with rs13946. When stratified by sex, significant associations for rs12722 were observed in females for both GR ( $P < 0.01$ ) and GJL ( $P = 0.05$ ), but no associations were observed in males ( $P = 0.37-0.48$ ). Females with the CT genotype for rs12722 exhibited greater GR ( $4.5^\circ \pm 4.2^\circ$ ) than those with either the CC or TT genotypes ( $2.2^\circ \pm 3.1^\circ$  and  $1.7^\circ \pm 2.8^\circ$ , respectively;  $P < 0.03$ ), and those with the CT genotype had a greater GJL score ( $2.5 \pm 1.8$ ) than those with the CC genotype ( $0.9 \pm 1.3$ ;  $P < 0.01$ ). However, GJL score was not different between the CT and TT genotypes ( $1.5 \pm 1.1$ ;  $P = 0.08$ ). Consistent with the inferences of a previous analysis,<sup>31</sup> the presence of the T allele in females was also associated with greater GJL in CT and TT ( $2.1 \pm 1.6$ ) genotypes versus the CC ( $0.9 \pm 1.3$ ) genotype ( $P < 0.01$ ). No association was seen for the whole or male groups ( $P = 0.06$  and  $P = 0.73$ , respectively).

The male sample population for rs12722 was in HWE, while the female subset was not. The  $\chi^2$  analysis of race and genotype indicated that the CC genotype of rs12722 was significantly overrepresented and the TT genotype underrepresented in the black subgroup, indicating a potential bias in our results. To reduce the risk of false positives caused by population stratification, rs12722 was reanalyzed without the black subgroup. The association of rs12722 with GJL was not detected in the nonblack subpopulation, although a trend still existed ( $P = 0.07$ ). When stratified by sex, significant associations remained in females for both GR ( $P = 0.02$ ) and GJL ( $P = 0.02$ ) but, as before, was not seen in males ( $P = 0.59-0.86$ ). Females with the CT genotype for rs12722 exhibited greater GR ( $4.6^\circ \pm 4.3^\circ$ ) than those with either the CC or TT genotype (respectively,  $1.7^\circ \pm 2.3^\circ$ ,  $P = 0.01$ ; and  $1.7^\circ \pm 2.8^\circ$ ,  $P = 0.02$ ) and had a greater GJL score ( $2.4 \pm 1.8$ ) than those with the CC genotype ( $0.9 \pm 1.3$ ;  $P < 0.01$ ) but not the TT genotype ( $1.5 \pm 1.1$ ;  $P = 0.10$ ). Nonblack females with the T allele of rs12722 had significantly higher GJL scores (CT + TT;  $2.0 \pm 1.6$  vs CC;  $0.9 \pm 1.3$ ;  $P = 0.02$ ), but these differences were not observed in the whole or male group analyses ( $P = 0.15$  and  $P = 0.77$ , respectively).

We noted a trend for the combined sex and female subsets for rs970547 within the coding region of *COL12A1* where all laxity scores tended to be lower for the GG and AG genotypes compared to AA ( $P = 0.1$ ). As done in a prior analyses of this variant with ACL injury,<sup>30</sup> we combined the GG and GA genotypes and compared these to the AA genotype. This resulted in a significant association for AKL when females and males were combined ( $P = 0.03$ ) and within the female subset ( $P = 0.03$ ) but not for the male subset ( $P = 0.64$ ). Specifically, females with the AA genotype had greater AKL ( $6.7 \pm 1.9$  mm) than the combined AG and GG genotypes ( $5.8 \pm 2.07$  mm). This result suggests that the presence of the G allele is associated with lower magnitudes of AKL.

No genotypic associations were observed for any of the laxity variables for rs240736 within *COL12A1* when males and females were analyzed together ( $P = 0.10-0.90$ ) or when females were analyzed separately ( $P = 0.06-0.87$ ). In the male subset, the CC genotype for rs240736 exhibited greater GJL ( $3.0 \pm 1.4$ ) than both the CT and TT genotypes ( $0.6 \pm 0.9$  and  $1.1 \pm 1.4$ , respectively;  $P < 0.04$ ). These results should be interpreted with caution, however, as only 2 males carried the CC genotype. Both *COL12A1* SNPs were in HWE for all subjects and did not show any racial bias.

Table 3 presents a comparative summary of the genetic associations with joint laxity (current study) with prior reports of genetic associations with ACL injury.<sup>18,29-31</sup>

**Table 3.** Comparative findings of associations with target single-nucleotide polymorphisms and joint laxity versus anterior cruciate ligament (ACL) injury.

Gene	Polymorphism	Associations With Laxity	Associations With ACL Injury
COL1A1	rs1800012	TG + TT genotypes were associated with greater GR vs the GG genotype (all subjects)	TT genotype was underrepresented in ACL injured vs controls <sup>18,29</sup>
COL5A1	rs12722 rs13946	CC genotype showed decreased GR and GJL than the CT genotype, and the CT showed greater GR vs the TT in females only No association	CC genotype was underrepresented in ACL-injured females compared to controls <sup>31</sup> No association with ACL injured <sup>31</sup>
COL12A1	rs240736 rs970547	CC genotype (n = 2) was associated with greater GJL vs the CT or TT genotypes in males AA genotype was associated with greater AKL vs the GA + GG genotypes in females only	No association with ACL injured <sup>30</sup> AA genotype was overrepresented in ACL-injured females and females with family history of ACL injury compared to GA + GG <sup>30</sup>

## Discussion

This is the first report to our knowledge investigating genetic polymorphisms associated with ACL injury history<sup>18,29-31</sup> on specific joint laxity measures that have also been associated with ACL injury history.<sup>19,23,26,32,34,43,46</sup> These are 2 ACL injury risk factors that to date have been considered independent of each other. The consistency in our findings with joint laxity scores compared to previous studies examining the relative frequency of these genotypes in ACL-

injured populations largely confirms our hypothesis that genotypes associated with a history of ACL injury are also associated with increased joint laxity measures.

Specifically, the CC genotype of rs12722 and the AA genotype of rs970547, which were previously associated with decreased and increased injury risk, respectively,<sup>30,31</sup> were also associated with lesser and greater magnitudes of joint laxity, respectively. Moreover, the female-specific genetic associations we observed for joint laxity for rs12722 and rs970547 are consistent with female-specific associations with ACL injury.<sup>30,31</sup> In addition, we observed no association with joint laxity for SNPs that were not associated with ACL injury (rs13964 and rs240736),<sup>30,31</sup> suggesting that there are specific genetic changes in collagen genes that increase ACL injury risk by altering the amount and structure of collagen proteins.

### **Relationship Among Collagen, Knee Laxity, and ACL Injury Risk**

Given the hierarchical nature of collagen fibers,<sup>13</sup> the importance of individual molecular bonds on strain capabilities of collagen,<sup>44</sup> and the interactions between collagen types,<sup>13</sup> small changes in the amino acid sequence or abundance of these proteins will likely affect the overall structure and function of a ligament. In molecular models of type I collagen, changes in the amino acid sequence and the presence of cross-links between fibers alter the biomechanical properties of the structure.<sup>44,45</sup> This work directly supports a previous hypothesis that density of cross links effects biomechanical capabilities.<sup>12</sup>

A first look into the genetic variants within the *COL5A1* gene reveals that the T allele of rs12722 confers greater messenger RNA stability than the C allele<sup>20</sup> and presumably increases the amount of *COL5A1* protein produced in the cell. This difference, in turn, has been hypothesized to alter fibrillogenesis and overall collagen structure.<sup>7,20</sup> Type V collagen is known to interact with type I collagen to form a basic subunit upon which further collagen molecules build upon; thus, type V potentially represents an important component of the core of a fiber.<sup>3,13</sup> The altered abundance of type V protein may change the biochemical and thus biomechanical properties within the ACL that have been measured physiologically. In an ACL-injured group, this CC genotype has been shown to be underrepresented<sup>31</sup> and is further associated with lower GR and GJL scores in females in this study, both of which implicates the CC genotype as decreasing joint laxity and injury risk.

For rs970547, prior work has shown that the AA genotype was overrepresented in the ACL-injured participants versus controls when compared to the combined AG + GG genotype.<sup>30</sup> This finding is consistent with our finding that the AA genotype increased AKL when compared to the AG + GG genotypes. These results are biologically plausible, as the amino acid position that is altered by rs970547 (glycine to serine at position 3058 in the protein sequence; G3058S)<sup>27</sup> lies within the NC1 functional binding domain,<sup>42</sup> which is thought to be essential for collagen matrix organization.<sup>17</sup> This NC1 domain is thought to protrude out of the main structure and provide an interaction site for extracellular matrix molecules and potentially other collagen fibers.<sup>17</sup> If this



amino acid change alters the binding domain significantly, the altered collagen structure may be presented clinically through joint laxity and ultimately in ACL rupture.

Our association with elevated GR and the T allele of rs1800012 was inconsistent with previous findings<sup>29</sup> but may be explained through the T variant's association with other pathologies, such as lower bone quality and density.<sup>24</sup> In bone, the T substitution increases Sp1 binding and mRNA expression, which in turn results in a greater ratio of  $\alpha 1(I)$  relative to  $\alpha 2(I)$  (the primary structural proteins of type I collagen).<sup>24</sup> In turn, this has been associated with lower bone quality and density and reduced fracture strength.<sup>24</sup> While the implications of increase in the  $\alpha 1(I)$  chain relative to the  $\alpha 2(I)$  chain on ligament density and strength is unknown, these properties could have an impact on knee joint laxity. In summary, as molecular differences have been implicated to change collagen fiber biomechanics,<sup>44,45</sup> overall differential collagen organization may be explained through previously unrelated measures of ligament behavior. Joint laxity provides a starting point for such investigation.

### **Sex-Specific Associations**

The female-specific genetic associations observed between joint laxity and both rs12722 and rs970547 are consistent with the female-specific genetic associations with ACL injury for these SNPs.<sup>30,31</sup> These associations may reflect a potential hormone-gene interaction,<sup>30</sup> given the known effects of sex hormones on collagen metabolism<sup>49</sup> and the potential for estrogen to interact with mechanical tension to differentially regulate gene expression and, thus, the abundance of collagen.<sup>21</sup> It is known that GR, GJL, and AKL are greater in females compared to males<sup>38</sup> and can vary with hormone concentration changes across the female's menstrual cycle.<sup>37</sup> In an effort to better understand these sex-specific findings, further mechanistic work is needed to determine how sex hormones influence the expression of candidate genes associated with collagen regulation and how genotypic differences affect these hormonal responses.

### **Conclusion**

Collectively, our results strengthen a growing body of work that indicates a genetic influence on ACL injury risk. Specifically, these data provide insight into one potential pathway through which genotypic variants in collagen genes have the potential to alter the structure and behavior of ligaments restraining joint motion (thus, joint laxity), thereby influencing injury risk. Moreover, the relative consistency in the associations between these collagen gene variants with both joint laxity and ACL injury risk suggests that screening an individual's joint laxity may have prognostic value in determining an individual's susceptibility to injury risk and initiating tailored intervention strategies to mitigate this risk. However, substantially more work is needed to fully characterize the interaction of these collagen gene variants with ligament structure and function and, ultimately, their effects of knee joint laxity and ACL injury risk.

There are several limitations to this study. Because of the hypothesis-driven nature of this study, the sample size is relatively small. Only 5 SNPs were studied, and given the number of genes

that have the potential to influence the ligament structure, additional relevant genetic variants should be explored. Also, the genotypic frequencies for rs1800012 and rs12722 are disproportionately represented, which is likely explained through our exclusion of previously injured individuals. That is, our healthy sample tended to have lower proportions of genotypic variants (Table 2) that have been associated with ACL injury.<sup>29,31,35</sup> It will be important to examine these genetic associations in larger populations to validate and refine these findings.

### Footnotes

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