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Type I Collagen α1 Sp1 Polymorphism and the Risk of Cruciate Ligament Ruptures or Shoulder Dislocations

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Background: Cruciate ligament ruptures and shoulder dislocations are often caused by trauma, but predisposing intrinsic factors might also influence the risk. These injuries are more common in those with a previously injured sibling, an observation that might indicate a genetic predisposition. It is well known that polymorphisms in the collagen I gene are associated not only with osteoporosis and osteoporotic fracture risk, but also with osteoarthritis.

Hypothesis: Because collagen I is abundant in ligaments and tendons, the authors hypothesized that collagen I α1 Sp1 polymorphism also was related to the occurrence of cruciate ligament ruptures and shoulder dislocations.

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

Methods: A total of 358 patients and 325 randomly selected population-based female controls were included in the study. Of the cases, 233 had a cruciate ligament rupture and 126 had had a shoulder dislocation. Age-adjusted odds ratios (ORs) with 95% confidence intervals (CIs) estimated by unconditional logistic regression were used as measures of association.

Results: Compared with the homozygous SS category, the heterozygous participants displayed a similar risk (OR, 1.06; 95% CI, 0.76-1.49), whereas the ss genotype was underrepresented in the injured population compared with the controls (OR, 0.15; 95% CI, 0.03-0.68). This latter estimate was similar for both cruciate ligament ruptures and shoulder dislocations, and was furthermore not modified by general joint laxity.

Conclusion: The authors found a substantially decreased risk of these injuries associated with collagen type I α1 Sp1 polymorphism. The study might encourage other investigators to consider further research in the area of genes and soft tissue injuries.

Keywords: cruciate ligament rupture; shoulder dislocation; polymorphism; gene; collagen

Cruciate ligament ruptures are always caused by a trauma, which is often also the case for shoulder dislocations. The annual incidence of cruciate ligament injuries is 1 per 10 000 athletes.[22] The incidence of shoulder dislocations is 8 per 100 000 person-years.[28] Even though these injuries are normally caused by accidents, predisposing intrinsic factors might influence risk. It is a clinical observation that the risks of both cruciate ligament injuries of the knee and shoulder dislocations are higher for subjects with siblings previously affected by these injuries.[4,5] These observations could be due to common vigorous lifestyles in affected families, but also to genetic constitution that predisposes the individual to soft tissue injuries.

Genetic variants of ligament tissue matrix proteins have not previously been examined in association with acute soft tissue injuries. Collagen type I fibrils are a major constituent of bone matrix and form strong parallel bundles of fibers in tendons and ligaments. The major 2 genes that regulate collagen production are the collagen Iα1 (*COLIA1*) and the collagen Iα2 (*COLIA2*) gene. The *COLIA1* and *COLIA2* encode collagen Iα1 and collagen Iα2 polypeptides, respectively, which associate in a 2:1 ratio to form collagen type I. Control of this process is at the transcriptional level. Mutations and single nucleotide polymorphisms in the gene, on chromosome 17, coding for collagen Iα1 chain have been associated with variations in bone structure as

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well as with bone mineral density (BMD). In addition, mutations in *COLIA1* results in osteogenesis imperfecta, a disease characterized by a fragile collagen structure leading to multiple skeletal fractures but which can also involve ligaments, tendon, and teeth.[7,23] Polymorphism in the promotor region of intron 1 of *COLIA1*, a predicted binding site for the transcription factor Sp1, is reported to be associated with BMD and fracture risk.[1,34] Substitution from guanine (G) to thymine (T) in *COLIA1* intron 1 is referred to as "s," and considered unfavorable with respect to BMD, whereas "SS" indicate homozygosity for GG.[34] Low back pain in young military recruits is also more common in those with the ss genotype than in age-matched controls.[33] On the contrary, osteoarthritis is less common among those with the ss genotype.[13,16]

Because collagen type I is a major protein constituent of cruciate ligaments, joint capsules, and tendons, we hypothesize that a polymorphism in *COLIA1*, associated with lower BMD and osteoarthritis, might well be associated with soft tissue injuries. To address this question, we genotyped the collagen Iα1 Sp1 polymorphism in deoxyribonucleic acid (DNA) samples from patients suffering from damaged cruciate ligaments or shoulder dislocations and compared these allele frequencies with those from a healthy population-based control group. Explicitly, because osteoarthritis is known to be associated with these soft tissue injuries[15,19] and osteoarthritis is associated with lower frequency of the ss genotype, we postulate that this genotype also might be associated with a reduced risk of the soft tissue injuries.

PARTICIPANTS AND METHODS

Participants

All patients treated at the orthopaedic department of Uppsala University Hospital for an arthroscopically established cruciate ligament rupture or radiologically confirmed shoulder dislocation were invited to participate in our study. The enrollment took place from 1999 through 2003. In total, 358 patients (age, 15-60 years) accepted, whereas only 3 declined participation. Of the 358 cases, 233 suffered from damage of the cruciate ligament and 126 had had a shoulder dislocation; 1 patient had both diagnoses. Joint laxity according to Carter and Wilkinson was determined.[2] Leisure physical activity (none, <1 h/wk, 1-2 h/wk, or >2 h/wk) in recent years and as a teenager was determined by a questionnaire.

From a previously described cohort randomly selected from the population register,[10] 325 females with whole blood samples were used as a control group in the present investigation. They were aged from 19 to 39 years. We did not believe that this would pose a problem, because the gene for the collagen I type 1α is located on the autosomal chromosome 17, and not on the sex chromosomes. This collagen gene is therefore inherited in a similar way in both men and women. Even if the absolute number of an outcome was differently distributed in men compared with women, the relative distribution of the outcome, and thus the odds ratios, by the genotype is not affected. We compared the relative distribution of injuries by the genotypes in controls to that in cases, and this distribution is not affected by sex.

All participants had their weight measured on a scale and their height measured with a ruler. The Uppsala University ethics committee approved the study, and informed consent was obtained from all participants.

Genotyping for the Collagen I α1 Sp1 Polymorphism

Genomic DNA from each individual was extracted from 3 mL of whole blood using a Wizard Genomic DNA purification kit (Promega Corporation, Madison, Wisconsin). The genotype of each individual was determined using solid-phase minisequencing.[14,31,35] Briefly, a 152-bp fragment of the collagen Iα1 gene was amplified with a biotinylated forward primer (TCCAATCAGCCGCTCCCA) and a reverse primer (GGGA GGGCAGGCTCGTG). Polymerase chain reactions (PCRs) were run on a Gene Amp PCR system-9700 robot using Ampli-Taq Gold kits and standard reagents (Perkin Elmer Co, Norwalk Connecticut). The amplification profile consisted of denaturation at 96°C for 15 minutes, followed by 36 cycles with denaturation at 96°C for 30 seconds, annealing at 62°C for 30 seconds and elongation at 72°C for 1.5 minutes, and final extension at 72°C for 7 minutes. The PCR products were captured in streptavidin-coated microtiter plates and rendered single-stranded. The polymorphic nucleotide was detected in the captured DNA strand by single-base extension of the primer GTCCAGCCCTCATCCCGCCC with ^{3}H-labeled nucleotides, the primer anneals immediately adjacent to the polymorphic site. The genotype of the individual is defined by the ratio between incorporated ^{3}H-labeled nucleotides.

Statistics

The injury risk associated with the 3 genotypes of the collagen Iα1 gene was analyzed with the SS genotype as the reference. For these associations, we used age-adjusted unconditional logistic regression to estimate odds ratios (ORs) and 95% confidence intervals (CIs). We furthermore considered to additionally include body weight and height, or body mass index, in the age-adjusted model but with only marginal influences on our estimates. Consequently, we only present results from the age-adjusted model, with age in continuous form. Statistical analysis was carried out using SAS software (version 9.1, SAS institute Inc, Cary, North Carolina).

RESULTS

Clinical characteristics of the subjects by genotype and sex are presented in Table 1. The control group consisted only of females, whereas the case group is presented according to sex. The genotype distribution among controls displayed that 71% were SS, 25% were heterozygotes Ss, and only 4% were ss. The polymorphism genotyped in the study cohorts was in Hardy-Weinberg equilibrium. (Hardy-Weinberg equilibrium is a common test in genetic

TABLE 1
Clinical Characteristics (Mean ± Standard Deviation) for Controls and Cases (Cruciate Ligament Injury or Shoulder Dislocation) by Procollagen Iα1 Genotype

Characteristic	SS	Ss	ss
Controls, female			
n	230	83	12
Height (cm)	167.8 ± 5.9	167.0 ± 6.3	165.6 ± 5.1
Weight (kg)	66.7 ± 13.1	67.5 ± 12.0	63.2 ± 9.6
Age (y)	29.5 ± 5.9	29.14 ± 5.5	31.2 ± 7.8
Body mass index	23.6 ± 4.4	24.2 ± 4.4	23.0 ± 2.5
Cases, female			
n	119	41	1
Height (cm)	168.5 ± 6.5	169.0 ± 6.5	167
Weight (kg)	67.3 ± 10.6	65.9 ± 10.7	65
Age (y)	27.0 ± 9.5	26.9 ± 7.9	50
Body mass index	23.6 ± 3.3	23.1 ± 4.0	23.3
Cases, male			
n	138	58	1
Height (cm)	180.0 ± 6.7	180.2 ± 6.6	190
Weight (kg)	79.2 ± 11.5	79.5 ± 13.1	92
Age (y)	28.6 ± 7.7	26.8 ± 8.2	19
Body mass index	24.4 ± 2.8	24.5 ± 3.6	25.5

TABLE 2
Age-Adjusted Odds Ratios With 95% Confidence Intervals (CI) of Having a Cruciate Ligament Injury or a Shoulder Dislocation According to Procollagen Iα1 Sp1 Genotype

Injury	Genotype	Cases	Controls	Odd Ratio (95% CI)
All	SS	257	230	1.0
	Ss	99	83	1.06 (0.76-1.49)
	ss	2	12	0.15 (0.03-0.68)
Cruciate ligament rupture	SS	162	230	1.0
	Ss	70	83	1.19 (0.82-1.75)
	ss	1	12	0.12 (0.02-0.92)
Shoulder dislocation	SS	95	230	1.0
	Ss	30	83	0.88 (0.54-1.41)
	ss	1	12	0.20 (0.03-1.56)

epidemiology. It measures the chance of a skewed genotype distribution. If the genotype distribution is not in Hardy-Weinberg equilibrium, there is a risk of selection bias in the study.) Cases and controls had a similar age distribution. Female and male cases had a similar genotype distribution, both with only 1 case with the ss genotype. The anthropometric measures were not associated with the genotypes (all $P > .3$).

There was a substantial 85% reduced risk (95% CI, 34%-97%) of injury for those with the rare ss genotype compared with those with presence of the genotype SS (Table 2). We observed a similar reduction in risk for cruciate ligament ruptures and shoulder dislocations. No significant difference in injury risk was observed among those with the Ss genotype compared with the homozygotes with the SS genotype. Within the control group, there were 12 cases with former knee injuries. Exclusion of these cases did not affect our estimates (data not shown). Furthermore, excluding 101 cases with clinical joint laxity according to Carter and Wilkinson revealed no attenuation in risk for those with the ss genotype (OR, 0.10; 95% CI, 0.01-0.81). We found no association between recent or teenage leisure physical activity level and genotype among the cases (Fisher exact test; $P = .91$ and $P = .73$, respectively).

DISCUSSION

We found that the risk of cruciate ligament ruptures and shoulder dislocations is associated with a polymorphism in the *COLIA1* gene. Individuals with the rare genotype ss with 4% prevalence in our study were underrepresented in the injured group. This implies that they had a substantially reduced risk of these soft tissue injuries. This is a novel finding.

Type I collagen is the most abundant protein in the body, and it is synthesized by fibroblasts, osteoblasts, and odontoblasts.[26] Although bones, ligaments, and joint cartilage contain this common collagen type, various reports have described different locations for the regulation of the promotor region that controls transcription of *COLIA1*.[26] One such location is the Sp1 transcription factor–binding site of the *COLIA1* gene. Functional analysis has shown that the osteoarthritis- and osteoporosis-related s allele of the Sp1 polymorphism is associated with increased DNA-protein binding, increased transcription from the s allele, and increased production of the collagen type Iα1 messenger ribonucleic acid (mRNA) and protein.[17] Type I collagen molecules consist of 2 α1 and α2 chains, which are synthesized in a 2:1 ratio. These are encoded by 2 different genes located on chromosomes 17 and 7 in humans. Type I collagen fibers have a high tensile strength and are resistant to most proteases.[26] Although type I collagen is essential to provide mechanical strength to tissues as exemplified by osteogenesis imperfecta,[29] its abnormal accumulation, along with other extracellular matrix proteins, is characteristic of fibrotic diseases.

Even though the s allele of the Sp1 polymorphism is associated with an increased production of collagen type I α1 chains, it is still unclear how the ss genotype would reduce the odds of having a soft tissue injury. Our results are in accordance with those for osteoarthritis[13] but not those for BMD.[25] There are associations between both cruciate ligament ruptures and shoulder dislocations with future osteoarthritis.[15,19] However, it is uncertain if the development of secondary osteoarthritis is also associated with collagen Sp1 polymorphism (ie, less likely to occur in those with the ss genotype). We speculate that the increased production of collagen type Iα1 mRNA and protein[17] also increases the tensile strength of tendons and ligaments. It has been proposed, in an effort to explain why an increased production of collagen confers an increased risk of osteoporosis, that the resulting imbalance between the α1 and α2 chains of collagen type I may contribute to impairment of bone strength measured by biomechanical testing.[17,24,30]

One of the most widely studied polymorphisms in relation to bone quality is the Sp1 polymorphism in the *COLIA1* gene,[32] which has been associated with low bone mass[9,11,37] and osteoporotic fracture risk in postmenopausal women[8] and female children.[27] A recent pooled analysis of more than 20 000 individuals confirmed that the *COLIA1* Sp1 binding site polymorphism was found to be significantly associated with BMD. Prior studies have displayed 30% increased fracture risk for the Ss genotype and 80% increased risk for the ss genotype compared with SS, with an even greater risk when the analysis was confined to vertebral fractures.[12,18] Collagen type I provides elasticity and flexibility to bone but also determines its structural organization. Thus, collagen type I binds and orients other proteins that nucleate hydroxyapatite deposition. Hydroxyapatite binds minerals such as calcium and phosphate, and these together form the crystal lattice of the bone. If the orientation of hydroxyapatite crystals is deranged by an overproduction of the collagen α1 compared with the α2 chains for the s allele, it might lead to a bone with impaired strength. Several studies have shown that the *COLIA1* genotype predicts osteoporotic fractures by a mechanism partly independent of BMD, indicating that polymorphism may act as a marker for bone quality as well as density.[11,18,20,34] The result of the overproduction of collagen type Iα1 in tendinous tissues might, however, result in higher tensile strength.

This polymorphism is not only associated with musculoskeletal diseases. Over 25% of women with cervical insufficiency have a family history of cervical insufficiency, and the *COLIA1* Sp1 binding site polymorphism has been associated with the condition.[36] Furthermore, otosclerosis, the single most common cause of hearing impairment in white adults, has also been associated with polymorphisms in the *COLIA1* gene.[3] In addition, Mokone et al[21] have investigated variants of the gene expressing another extracellular matrix protein, tenascin-C, and the risk of chronic Achilles tendinosis and ruptures by a case-control design. They found that a guanine-thymine repeat polymorphism was associated with these tendon injuries. The tenascin-C gene is located on chromosome 9, a different locus than that for the *COLIA1* gene.

The Sp1 polymorphism of the *COLIA1* gene, located on the autosomal chromosome 17, is equally distributed in women and men,[9] and this polymorphism has a recessive mode of inheritance.[22] The genotype distribution in our controls was similar to that in earlier reports,[22] and we found no difference in case distribution by genotype among female and male cases. Even if the absolute number of female cases (n = 161) were less than the number of male cases (n = 197), that does not affect our ORs because these are estimated by the relative distribution of cases to the relative distribution of controls by the genotypes.

Our study has some strengths and some conceivable limitations. Only 3 out of all our possible cases declined to participate in the study. We also had the opportunity to compare our cases with population-based controls. Thus, selection on gene status is unlikely. Because the genotype ss with the aberrant risk of injury was uncommon, the reduced risk might have occurred by chance even if it was statistically significant. However, this uncommon but functional genotype has consistently been reported to either increase or decrease risk of several different outcomes.[6,17,33,35] Even though the genotype ss has been found to be associated with low bone density in the elderly, we found no bone injuries among those with this genotype.

In conclusion, we found that the *COLIA1* Sp1 ss genotype was associated with a substantially reduced OR of cruciate ligament ruptures and shoulder dislocations. As our influential genotype is rare, the absolute risk of cruciate ligament injuries and shoulder dislocations seem to be only marginally affected by COLIA1 Sp1 polymorphism. Accordingly, this particular single nucleotide polymorphism has limited clinical relevance as a predictor for these injuries. Our study might encourage other investigators to consider further research in the area of genes and soft tissue injuries.

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