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Genetic variation in candidate genes and patellar tendinopathy: Prospective cohort study of 126 elite volleyball players

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Abstract

Variation in genes coding for structural proteins may represent risk factors for developing tendinopathy among athletes. The purpose of this prospective cohort study of elite volleyball students was to investigate whether specific single nucleotide variants (SNVs) in selected candidate genes, known to be associated with Achilles tendinopathy, were associated with the risk for developing patellar tendinopathy (jumper′s knee). Of 126 Caucasian athletes (64 females and 62 males), 33 cases (athletes developing jumper′s knee) were compared to 93 healthy controls. Six SNVs, distributed in the candidate genes COL1A1, COL5A1, MMP3, and GDF3, were genotyped. Baseline characteristics, genotypes, and minor allele frequencies (MAF) were compared between groups. Neither genotype nor minor allele frequencies differed significantly between the jumper′s knee group and the healthy controls. However, the low-frequency homozygous T/T genotype of the COL1A1 gene (rs1800012) was absent in the jumper′s knee group (P = .075). Separating the two study groups by gender suggested that there may be a female-specific genotype pattern, although the sample was too small for statistical calculations. In this study, although limited by sample size, we could not detect any clear relationship between six selected SNVs located in candidate genes and the risk for the development of jumper′s knee in elite volleyball students.

INTRODUCTION

The prevalence of jumper's knee (patellar tendinopathy) is high, up to 40%-50%, in sports characterized by high demands on leg extensor speed and power, such as volleyball, basketball, football, and athletics.[1, 2] Several risk factors have been investigated, and tendon load seems to be a key element.[2-7] However, the complex process from a healthy tendon to jumper's knee is not fully understood.[8] Load tolerance and risk for tendon injuries vary between athletes.[9] Thus, interindividual differences, due to variation in genes coding for different musculoskeletal tissue and structure proteins, may affect the risk for developing patellar tendinopathy.

The COL1A1 gene encodes for a type I collagen forming heterotypic fibers in tendons with type II or V collagens coded by the COL2A1 and COL5A1 genes, respectively. Functional sequence variants in these structural genes could play important roles in regulating fibrillogenesis and therefore tendon strength.[10, 11] Genetic association studies of tendinopathy are relatively sparse, but single nucleotide variants (SNVs) within the COL1A1, COL5A1, MMP3, and TNC genes, encoding collagens, metalloproteinase 3 and tenascin C, respectively, have been shown to be linked to Achilles tendinopathy and rupture of the Achilles tendon.[9, 12, 13] Genetic research on patellar tendinopathy is even more limited; the only study available is a case-control study with different types of tendon diseases.[14]
The purpose of this prospective cohort study was therefore to investigate whether specific SNVs in selected candidate genes, known to be associated with Achilles tendinopathy, are associated with the development of jumper′s knee.

MATERIALS AND METHODS

Participants

Participants, all Caucasians of northern European origin, were recruited from a 5-year prospective cohort study of elite junior volleyball athletes attending a 3-year combined training and boarding high school program (ToppVolley Norge, TVN) in Norway. The students started at the age of 15-16 years, and they were expected to complete 3 years for a college-entry baccalaureate degree. Some students entered the program in the second or third year directly. All attending students gave their written informed consent to take part in the cohort study, which was approved by the Regional Committee for Medical and Health Research Ethics and the Norwegian Social Science Data Services. Parental consent was also obtained.

Diagnosis

The end point was a clinical diagnosis of jumper's knee. The following diagnostic criteria were used: (a) a history of pain in connection with training or competition in the quadriceps or patellar tendons at their patellar insertions; and (b) tenderness to palpation corresponding to the painful area.[15, 16, 2, 17] Players fulfilled the criteria to have jumper's knee when they reported the presence of symptoms for a minimum of 12 weeks and considered the symptoms to represent a substantial problem.

Genetic testing

Based on previous studies on Achilles tendinopathy, relevant candidate genes were selected for association studies of known SNV sites and development of jumper′s knee. In total, six selected SNVs distributed in the COL1A1 (rs1800012), COL5A1 (rs12722), MMP3 (rs591058, rs650108, rs679629), and GDF3 (rs143383) genes were analyzed.

DNA isolation
EDTA whole blood was collected from all participants at time of income to TVN and stored at −20°C until analyzed. Genomic DNA was extracted using a MagNA Pure LC DNA Isolation kit I (cat# 03 003990 001, Roche, Basel, Switzerland) on a MagNAPure LC Instrument (Roche). DNA yield and purity were controlled using Nanodrop 1000 Spectrophotometer (Thermo Scientific, Waltham, USA) before storage at +4°C prior to downstream analysis.

Genotyping
All the selected SNVs were genotyped by PCR using allelic discrimination with predesigned, specific TaqMan SNP Genotyping assays (Thermo Fisher Scientific, Life Technologies, Carlsbad, CA, USA) on a Viia7 Real-Time PCR Instrument (Thermo Fisher Scientific) essentially as recommended by the manufacturer. PCR was conducted with 12.5 μL 2xTaqMan Universal PCR Master Mix (Thermo Fisher Scientific, Cat. No. 4304437), 1.25 μL TaqMan® SNP Genotyping Assay, 5 μL genomic DNA, and 6.25 μL DNase-free water. A non-template control and longitudinal controls, representing the three different genotypes for each SNV, were included in each run.

Statistical analyses
Statistical analyses were performed using SPSS 21.0 software (SPSS Inc., Chicago, IL, USA). Categorical variables (SNV and allele frequencies and baseline characteristics of the two study groups) were compared using Pearson chi-square test. Each individual SNV was tested for Hardy-Weinberg equilibrium (HWE) using Michael H. Court′s online calculator http://www.tufts.edu/~mcourt01/Documents/Court%20lab%20-%20HW%20calculator.xls.

RESULTS
During the 5-year study period, 126 athletes evenly distributed in gender (64 females and 62 males) were recruited. Height, weight, and age did not differ between groups (Table 1). Of the 126 athletes, 33 were diagnosed with jumper's knee, including 9 females (14%) and 24 males (39%).

Table 1. Baseline characteristics of the study participants

	Men		Women	
	Healthy (n = 38)	**Jumper's knee (n = 24)**	**Healthy (n = 55)**	**Jumper's knee (n = 9)**
Height (cm)	186 ± 6	189 ± 7	171 ± 6	172 ± 8
Weight (kg)	74 ± 9	77 ± 8	65 ± 8	65 ± 8
Age (year)	16.8 ± 0.8	16.9 ± 0.4	16.8 ± 0.4	16.8 ± 0.4

- Data are shown as the average ± SD.

The allelic distributions of the six SNVs selected, localized in the COL1A1, COL5A1, MMP3, and GDF3 genes, followed the HWE, both in the overall sample population and in either study group. Genotype and minor allele frequencies (MAF) of all sequence variants for the jumper′s knee and healthy control groups are shown in Table 2. None of the variants was significantly associated with jumper′s knee. However, the asymptomatic control group showed a 6% prevalence of the homozygous, low-frequent T/T genotype in the COL1A1 gene variant rs1800012, whereas this genotype was absent among all athletes included in the jumper′s knee group, although MAF were almost identical between the two groups (P = .67). The remaining SNVs genotyped displayed only faint allele frequency differences between the jumper′s knee and the healthy control groups (Table 2), and all MAF values were close to the reference frequencies reported for Caucasians (http://www.ncbi.nlm.nih.gov/snp/).

Table 2. Genotype distribution and minor allele frequencies (MAF) of selected SNVs in four candidate genes within athletes who have developed jumper′s knee or not

Gene	SNV	Genotype	Jumper′s knee (n = 33)	Healthy controls (n = 93)	*P* -value[a]	
					SNV genotype	MAF frequency
COL1A1	rs1800012 (G>T)	G/G	0.58	0.69		
		G/T	0.42	0.25		
		T/T	0	0.06		
		MAF (T)	T = 0.21	T = 0.19	.075	.67
COL5A1	rs12722 (C>T)	C/C	0.12	0.19		
		C/T	0.61	0.53		
		T/T	0.27	0.28		
		MAF (C)	C = 0.42	C = 0.46	.60	.67
MMP3	rs591058 (C>T)	C/C	0.21	0.21		
		C/T	0.64	0.55		
		T/T	0.15	0.24		
		MAF (C)	C = 0.53	C = 0.49	.56	.57
	rs650108 (A>G)	A/A	0. 03	0.04		
		A/G	0.39	0.38		
		G/G	0.58	0.58		
		MAF (A)	A = 0.23	A = 0.23	.94	.95

Gene	SNV	Genotype	Jumper′s knee (n = 33)	Healthy controls (n = 93)	*P* -value[a]	
					SNV genotype	MAF frequency
GDF5	rs679629 (A/G)	A/A	0.15	0.24		
		A/G	0.64	0.57		
		G/G	0.21	0.19		
		MAF (G)	G = 0.53	G = 0.48	.59	.47
	(rs143383) (C>T)	C/C	0.18	0.17		
		C/T	0.36	0.44		
		T/T	0.46	0.39		
		MAF (C)	C = 0.36	C = 0.39	.73	.68

- [a] Pearson chi-square test was used to compare SNV genotype and MAF frequencies between athletes with jumper′s knee and healthy controls.

Separation of the jumper's knee group and healthy controls by gender, the men and female genotype patterns of the SNVs varied. Genotype frequencies showed consistent levels among the two male study groups, whereas the female genotype pattern for all SNVs varied between the two groups, though generating too small sample sizes for statistical calculations (data not shown).

DISCUSSION

The main finding of this prospective cohort study on gene variants associated with jumper's knee was that six SNVs in the COL1A1, COL5A1, MMP3, and GDF-3 genes, previously found to be associated with Achilles tendon pathology, do not seem to be linked with jumper's knee to the same degree. Genotyping of all participants demonstrated that none of the SNVs studied were found to be associated with jumper′s knee, as neither genotype nor allelic frequencies differed significantly between the two groups. However, interestingly, the homozygous T/T genotype in the rs1800012 SNV of the COL1A1 gene, albeit rare in population samples, was completely absent in the jumper's knee group. In the present cohort, none of the 33 athletes with jumper′s knee were homozygote carriers of the T-allele, whereas

6% of the 93 controls were homozygous T/T. Despite a trend for COL1A1 genotype to differ between healthy controls and the jumper's knee group, our study did not detect a significant pattern difference. However, it should be acknowledged that the results must be interpreted with caution, as the sample size was limited to 33 cases. If the observed effect size (6% vs 0%) represents the effect size among volleyball players in general, this present study had insufficient power to detect it.

The G to T base transition located within intron 1 of the COL1A1 gene (chromosome 17) has been proposed as a functional sequence variant, and several case-control studies have examined whether this variant is associated with tendon disorders, such as anterior cruciate ligament and Achilles tendon ruptures.[18-21] The role in overuse injuries and tendinopathy is less documented, and the variant has never been examined for a potential association with patellar tendinopathy. Nevertheless, all of the studies referred above found the homozygous T/T group to be underrepresented in the injured study groups. Corresponding results were also shown in a study of patients with cruciate ligament ruptures and shoulder dislocations by.[22] To conclude, our observations regarding the rs1800012 SNV are in line with those of Posthumus et al[20, 23] and indicate that the presence of a double T-allele in the COL1A1 gene variant may protect against tendon disorders.

Mokone et al[13] have reported that the C>T variant (rs121722) in the promoter region of the COL5A1 gene (chromosome 9) is associated with chronic Achilles tendinopathy; however, the present study could not demonstrate any risk of jumper's knee associated with this base substitution. In addition, the same group has elaborated the over-representation of the homozygous C/C genotype in asymptomatic participants, which is in line with our results as well as data from other groups.[24] In the shelter of these observations, skewed genotype patterns between tendon patients and healthy controls are exposed for both the COL1A1 and the COL5A1 genes.

Several SNVs (rs591058, rs650108 and rs679629) in the MMP3 gene (chromosome 11) have been reported to be associated with Achilles tendinopathy,[9, 25] whereas neither patellar tendon properties[26] nor our results on patellar tendinopathy were found to be influenced by the same variants.

Athletes carrying the T/T genotype in the GDF5 gene (rs143383) may have twice the risk of developing Achilles tendinopathy.[27] This association was not found for ACL ruptures.[28] Our data suggest that the frequency of homozygous T/T may be elevated in participants affected by patellar tendinopathy compared to healthy athletes, but the study did not have sufficient power to detect a difference of the observed size. Animal studies have shown that GDF5 supplementation has a positive effect on tendon healing,[29] but the role of GDF5s in patellar tendinopathy is unknown.

Recently, it was reported from a study on male elite volleyball players that sequence variants in the FGF3 and BMP4 genes are associated with different forms of tendinopathy.[14] Unlike the present study, consisting only of patellar tendinopathy carriers, Salles et al collected a heterogeneous study group of 52 cases, where 30 athletes had patellar tendinopathy only and 6 athletes suffered from combined tendinopathies (patellar and Achilles or shoulder). The value of generating well-characterized clinical study groups should not be underestimated; therefore, the present study has focused on patellar tendinopathy alone to be able to uncover genetic associations involved in this condition.

Men have two to four times higher risk for developing patellar tendinopathy than women,[2, 3, 5, 7] but so far this elevated risk has not been explained by genetic findings. However, in the present study, division into gender suggested that there may be genetic differences between men and women. Whereas the genotype frequencies showed a uniform pattern among males, genotype differences were observed between injured and healthy females (data not shown). This tendency was the case for all SNVs analyzed; however, this observation should be interpreted with care, as the sample sizes were too small for statistical calculations. Several research studies in the Achilles tendon field have presented gender differences for genetic factors that may predispose for risk. Posthumus et al[23] have reported that the homozygote C/C genotype in the COL5A1 gene was significantly underrepresented in 38 female participants with anterior cruciate ligament ruptures, but not in 91 male. Although study power was severely limited in the present study, given the few females with jumper's knee, our results mimicked those from Posthumus et al[23] No female with jumper′s knee was a homozygous carrier of the C/C genotype, indicating that also this sequence variant may be associated with a decreased risk of jumper′s knee.

It should be noted that data from the present cohort have identified several important risk factors for the development of jumper′s knee.[4-6] The strongest sports-related predictors were shown to be tendon load, expressed as jump height and the volume of volleyball training and match exposure. The hunt for genetic factors that may contribute to explain the development of jumper′s knee is in its infancy. The results from the present study suggest that the development of patellar and Achilles tendinopathy may not be associated with the same SNVs. There may be several reasons for this apparent discrepancy. First, although interesting trends are observed in this study, the study power is limited by the relatively small sample size, which should be borne in mind when interpreting our findings. A larger sample size is needed to validate the results and to avoid making a type II error. Secondly, the patellar and Achilles tendons have somewhat different architecture, location, and function, which should be taken into account when considering factors related to genetic regulation. Lastly, keeping in mind that the pattern of genetic variation is closely linked to ethnic background, results from previous genetic association studies in the field of tendinopathy are based on different populations (South African, Australian, British, and Caucasian) with different genetic makeup as a complicating factor.[19]

The obvious strength of the current study is that it is based on a homogeneous cohort of athletes followed prospectively for several years.

PERSPECTIVES

Jumper′s knee has a high prevalence among volleyball elite players. The importance of identifying individual risk factors for the development of jumper′s knee is well recognized, particularly in athletes attending sports characterized by high power and demands on leg extensor. Basically, more knowledge on genetic variations and their impact on individual risk for the development of tendinopathies are awaited. This small, prospective cohort study did not detect any clear relationship between six selected SNVs located in the candidate genes COL1A1, COL5A1, MMP3, and GDF3 and development of jumper's knee in a group of young elite volleyball players. This is in contrast to what has been indicated by previous case-control studies on Achilles tendinopathy. Therefore, further investigation with larger sample sizes is needed and complementary studies of other, alternative genetic variants may provide additional insight and lead to screening strategies to foresee genetic predisposition for tendon injury and identify athletes at risk for the development of jumper′s knee.

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