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THE +1245G/T POLYMORPHISMS IN THE COLLAGEN TYPE I ALPHA I (*COL1A1*) GENE IN POLISH SKIERS WITH ANTERIOR CRUCIATE LIGAMENT INJURY

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ABSTRACT: Objectives: The aim of this study was to examine the association of +1245G/T polymorphisms in the *COL1A1* gene with ACL ruptures in Polish male recreational skiers in a case-control study. Methods: A total of 138 male recreational skiers with surgically diagnosed primary ACL ruptures, all of whom qualified for ligament reconstruction, were recruited for this study. The control group comprised 183 apparently healthy male skiers with a comparable level of exposure to ACL injury, none of whom had any self-reported history of ligament or tendon injury. DNA samples extracted from the oral epithelial cells were genotyped for the +1245G/T polymorphisms using real-time PCR method. Results: Genotype distributions among cases and controls conformed to Hardy-Weinberg equilibrium (p=0.2469 and p=0.33, respectively). There was a significant difference in the genotype distribution between skiers and controls (p=0.045, Fisher's exact test). There was no statistical difference in allele distribution: OR 1.43 (0.91-2.25), p=0.101 (two-sided Fisher's exact test). Conclusions: The risk of ACL ruptures was around 1.43 times lower in carriers of a minor allele G as compared to carriers of the allele T.

KEY WORDS: anterior cruciate ligament (ACL) rupture, *COL1A1* gene, collagen, polymorphism, skiers

INTRODUCTION

The popularity of alpine skiing has significantly increased over the last few decades. Current sports technology allows for extreme racing manoeuvres at high speed. Modern slope designs often demand substantial risk taking and advanced skills. Consequently, alpine sports range amongst the most injurious tourist activities [12].

Skiing injuries account for a significant proportion of all sport-related injuries and among these lower extremity injuries account for the vast majority. In an analysis of the distribution of injuries during alpine skiing accidents, lower extremity injuries (39%) predominated followed by upper extremity injuries (34%) [10]. Polish skiers also experience worrying injuries of lower extremities (65%), mainly sprains in the knee joints [3]. The knee is the most commonly injured body part, and is also predominant among severe injuries [7]. Ninety percent of knee ligament injuries involve the anterior cruciate ligament (ACL) or medial collateral ligament (MCL) [21]. Alpine skiing is a high-risk sport for injuries to the ACL [6,11] because while descending a hill, a skier must resist large centrifugal forces at a high velocity, while the knees are positioned in postures that place the ACL at risk of injury [16]. The important roles of the anterior cruciate ligament regarding knee stability, physiological kinematics, and proprioception are unquestioned [18]. The ACL is a stable static structure with mechanoreceptors distributed across its surface and can regulate dynamic stability by means of the neuromuscular reflex. ACL deficiency causes deficits in proprioception and balance, a decrease in muscular strength and functional performance, and biomechanical modifications of the injured lower limb [26].

The dense fibrous connective tissue of the ACL is composed of a large amount of collagen fibres arranged in a hierarchal pattern, giving it high tensile strength [28]. Ligament fibroblasts produce a number of extracellular matrix (ECM) components, including collagen types I and III, decorin and fibromodulin. Type I collagen comprises 95% of the total ligamentous collagen, but smaller amounts of types III, V, XII, and XIV collagen are also present. The fibrillar type III collagen forms heterogeneous collagen fibrils with type I collagen, but may inhibit collagen fibril diameter growth. Surrounding the collagen fibrils is an organic substance consisting of proteoglycans and glycosaminoglycans. Two abundant proteoglycans within the ligament in-

clude decorin and fibromodulin. These proteoglycans bind type I collagen and regulate collagen fibrillogenesis [2].

Collagen, specifically collagen type I, is the most abundant protein in tendon tissue, forming 60–80% of its dry weight. Collagen type III is found in significantly lower abundance than collagen type I, but increased expression is an indicator of tendon injury. Collagen turnover, an important component of a healthy ECM, is regulated by a group of enzymes called matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) [27].

Collagen type I is a heterotrimer consisting of two alpha 1 chains and one alpha 2 chain. It is initially synthesized as a pro-alpha chain with a propeptide at each end (N-propeptide and C-propeptide) [1]. The proteins are encoded by the *COL1A1* and *COL1A2* genes [5]. The gene that encodes for the alpha 1 chain of type I collagen is located on chromosome 17q21 [30]. Mutations within this gene have been shown to cause connective tissue disorders such as osteogenesis imperfecta or Ehlers-Danlos syndrome [5], systemic diseases with scleral thinning, and myopia [30].

Single nucleotide polymorphisms (SNPs) in the collagen type I (*COL1A1*) gene have been shown to be associated with several complex connective tissue disorders. The G to T substitution in an intronic Sp1 binding site (rs1800012), resulting in increased affinity for the transcription factor Sp1, and increased gene expression, has been one of the most extensively investigated polymorphisms within this gene [5]. *COL1A1* has also been shown to be associated with an increased risk of shoulder dislocations [19], Achilles tendon ruptures and Achilles tendinopathy [5]. Studies have also shown an association of another polymorphism with the risk of ACL rupture [20]. Posthumus et al. found that a rare TT genotype of the Sp1 polymorphism was significantly underrepresented among participants with ACL ruptures [19]. All participants were genotyped for the *COL1A1* Sp1 binding site polymorphism (G/T; rs1800012). It was theorized that a variant allele T of the Sp1 binding site polymorphism enhances the binding of the transcription factor Sp1, thereby increasing *COL1A1* expression and the production rate of alpha-1 chains, resulting in alpha-1 homotrimer production.

The aim of this study was to examine the association of +1245G/T polymorphisms in the *COL1A1* gene with ACL ruptures in Polish male recreational skiers in a case-control study.

MATERIALS AND METHODS

Study subjects. A total of 138 male recreational skiers (27±2) with surgically diagnosed primary ACL ruptures, all of whom qualified for ligament reconstruction, were recruited for this study. The control group comprised 183 apparently healthy male skiers (26±3) with a comparable level of exposure to ACL injury, none of whom had any self-reported history of ligament or tendon injury.

Ethics Committee

The Pomeranian Medical University Ethics Committee approved the study and written informed consent was obtained from each participant.

Determination of COL1A1 genotypes

Genomic DNA was extracted from the oral epithelial cells using GenElute Mammalian Genomic DNA Miniprep Kit (Sigma, Germany) according to the manufacturer's protocol. Allelic discrimination of the *COL1A1* +1245G/T (rs1800012) polymorphic site was performed using TaqMan Pre-Designed SNP Genotyping Assays (Applied Biosystems, USA), including primers and fluorescently labelled (FAM and VIC) MGB probes for the detection of alleles. All samples were genotyped on a Rotor-Gene real-time polymerase chain reaction (PCR) instrument (Corbett, Australia). Thermal cycler conditions were as follows: an initial step at 95˚C for 5 min, followed by 45 cycles of denaturation at 94˚C for 15 s and anneal/extend at 60˚C for 1 min [13].

Statistical analysis

Any differences in genotype and allele frequency were analysed using χ^2 tests (or Fisher's exact test). Odds ratios (OR) with 95% confidence intervals (95%CI) were calculated. All calculations were performed using STATISTICA data analysis software system, version 10 (StatSoft, Inc., 2011; www.statsoft.com), except Hardy-Weinberg equilibrium, which was tested with the programming language and environment R (http://www.r-project.org). P values <0.05 were considered statistically significant.

RESULTS

The distributions of the *COL1A1* genotypes and alleles are given in Table 1. We did not observe a statistical difference in allele distribution: OR 1.43 (0.91-2.25), p=0.101 (two-sided Fisher's exact test).

TABLE I. ASSOCIATION BETWEEN +1245G/T (SP1, RS1800012) IN *COL1A1* AND ACL RUPTURES

Group	n	COL1A1 genotype			P*	COL1A1 allele		P
		GG	GT	TT		G	T	
InjuredACL skiers	138	90	46	2	0.046	226	50	0.101
		-65.2%	-33.3%	-1.5%		(81.9%)	(18.1%)	
Control	183	139	39	5		317	49	
		-76.0%	-21.3%	-2.7%		(86.6%)	(13.4%)	

Note: *GG vs. GT+TT. Data is given as n-values with percentages in parentheses.

The risk of ACL ruptures was around 1.43 times lower in carriers of a minor allele G as compared to carriers of the allele T. Genotype distributions among cases and controls conformed to Hardy-Weinberg equilibrium: p=0.2469 and p=0.33, respectively. Owing to the small number of observations, we used the "collapsing cells method" to determine the statistical significance of genotype distribution (i.e. we made the calculation GG vs. GT and TT). We found a significant difference in the genotype distribution between injured skiers and controls (p=0.045, Fisher's exact test).

DISCUSSION

The anterior cruciate ligament is necessary for normal knee stability and movement. Unfortunately, the ACL is also the most frequently injured ligament of the knee, with severe disruptions requiring surgical intervention [9]. ACL rupture affects the loading pattern at the joint interfaces and the stability of the knee, which leads to abnormal loading of articular cartilage during functional activities, and ultimately to articular cartilage degeneration and progressive osteoarthritis of the knee [26]. It is estimated that 27 million adults in the United States have clinical osteoarthritis, with knee osteoarthritis being the most prevalent form, affecting 28% of adults over age 45 and 37% of adults over age 60. The most significant cause of osteoarthritis in young adults is joint trauma including tears of the menisci or ligaments [29]. The incidence of knee osteoarthritis in patients with untreated ACL deficiency 11 years after ACL rupture is as high as 44% [26].

A skier's risk of injury depends on a considerable number of different factors [14]. Skiing injuries may result from an individual overestimating his or her abilities or from fatigue. [10]. Snow, slope and weather conditions, as well as altitude and low temperatures, are thought to influence the prevalence of knee injuries [22]. Analysis of menstrual history data revealed that recreational skiers in the preovulatory phase were significantly more likely to sustain an ACL injury than were skiers in the postovulatory phase [23]. Although intrinsic and extrinsic factors for ACL ruptures have been identified, the exact aetiology of this injury is not yet fully understood [17].

Some studies have also suggested a genetic predisposition to Achilles tendon ruptures and chronic Achilles tendinopathy, as well as to tears of the ACL [25]. It was previ¬ously shown that individuals who had a family history of ACL rupture exhibited 2-fold higher risk for ACL rupture [8]. In the present study, the aim was to examine the association of +1245G/T polymorphisms in the *COL1A1* gene with ACL ruptures in male skiers in a case-control study. There was a significant difference in the genotype distribution between skiers and controls, but there was no statistical difference in allele distribution. The risk of ACL ruptures was around 1.43 times lower in carriers of a minor allele G as compared to carriers of the allele T.

Three studies have suggested that the rare TT genotype of functional Sp1 binding site polymorphism within intron 1 of *COL1A1* is associated with infrequency of cruciate ligament ruptures (CL), shoulder dislocation (SD) and Achilles tendon ruptures [4]. Collins et al. found that similar genotype distributions were reported for the control and injury groups in all three studies [4]. The TT genotype, when compared to the control group (4.1%, n=24 of 581), was significantly under-represented in the (1) CL (0.3%, n=1 of 350, OR=15.0, P=0.0002), (2) CL and SD (0.4% TT genotype, n=2 of 476, OR=10.2, P<0.0001), and (3) CL, SD and Achilles tendon ruptures (0.4% TT genotype, n=2 of 517, OR=11.1, P<0.0001) groups. This combined analysis indicates that the TT genotype appears to be protected against acute soft tissue ruptures and should be incorporated into multi-factorial models determining risk for acute soft tissue ruptures [4].

Posthumus et al. sought to determine whether the functional Sp1 binding site polymorphism within intron 1 of the *COL1A1* gene is associated specifically with ACL ruptures in an independent population [19]. All Caucasian participants were genotyped for the *COL1A1* Sp1 binding site polymorphism (G/T; rs1800012). The rare TT genotype was significantly (p = 0.031, OR = 0.08, 95% CI <0.01 to 1.46) under-represented in the ACL group (0 out of 117, 0%), compared with the controls (6 out of 130, 4.6%) [19].

Similar results were obtained in studies conducted among patients with cruciate ligament rupture and shoulder dislocation. 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. Varying levels of risk of these injuries were found in association with collagen type I alpha 1 Sp1 polymorphisms [15].

The role of genetics in sport research increases with every passing year. Knowledge of the role of individual genes in the processes occurring in the human body can also be used in sport rehabilitation and injury prevention. Precise determination of genotypes at risk for acute or chronic diseases related to sport will probably allow changes in individual training plans to greatly minimize the risk of injury [24].

CONCLUSIONS

In conclusion, in our study the risk of ACL ruptures was around 1.43 times lower in carriers of a minor allele G as compared to carriers of the allele T.

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