Journal of Science and Medicine in Sport 22 (2019) 408–412

Contents lists available at ScienceDirect

Journal of Science and Medicine in Sport

journal homepage: www.elsevier.com/locate/jsams

Original research

Are *TNC* gene variants associated with anterior cruciate ligament rupture susceptibility?

Ewelina Lulińska-Kuklik[a], Mary-Jessica Nancy Laguette[b,*], Waldemar Moska[a], Magdalena Weber-Rajek[c], Krzysztof Ficek[d], Ryszard Puchala[e], Paweł Cięszczyk[f,g], Marek Sawczuk[a], Alison Victoria September[b], Agnieszka Maciejewska-Skrendo[f]

[a] *Faculty of Tourism and Recreation, Gdansk University of Physical Education and Sport, Poland*
[b] *UCT Research Centre for Health through Physical Activity, Lifestyle and Sport (HPALS), University of Cape Town, South Africa*
[c] *Department of Physiotherapy, Collegium Medicum Nicolaus Copernicus University, Poland*
[d] *Department of Medicine and Sport Physiotherapy, Academy of Sport Education in Katowice, Poland*
[e] *Department of Applied Physiology, Military Institute of Hygiene and Epidemiology, Poland*
[f] *Faculty of Physical Education, Gdansk University of Physical Education and Sport, Poland*
[g] *Faculty of Physical Education, Academy of Sport Education in Katowice, Poland*

ARTICLE INFO

Article history:
Received 9 May 2018
Received in revised form 5 September 2018
Accepted 9 October 2018
Available online 17 October 2018

Keywords:
Tenascin C
ECM glycoprotein
Knee ligament injury
Genetic predisposition
Risk modulation

ABSTRACT

Objectives: To investigate the role of inter-individual variations in a particular glycoprotein, *TNC*, and its potential contribution to anterior cruciate ligament (ACL) injury susceptibility in Polish Caucasian participants. ACL rupture is one of the most prevalent and severe knee injury that predominantly occurs during sports participation, primarily via a non-contact mechanism. Several polymorphisms in genes encoding glycoproteins either independently or as allelic combinations, modulate the risk of musculoskeletal soft tissue injuries. Specifically, the *TNC* rs1330363 (C > T), rs2104772 (T > A) and rs13321 (G > C) variants, independently or in haplotype combinations, were analysed in this context.
Design: Case–control genetic association study.
Methods: A group of 421 physically active, unrelated participants were recruited where 229 individuals with surgically diagnosed primary ACL rupture and 192 apparently healthy participants without any history of ACL injuries. Participants were genotyped for the above variants.
Results: Genotype and allele frequencies of *TNC* variants did not differ between cases and controls. Haplotype analysis revealed no association between *TNC* and predisposition to ACL rupture.
Conclusions: Our analyses did not reveal a significant association between these *TNC* variants and risk of ACL rupture in Polish Caucasian participants.

1. Introduction

Ligaments and tendons are believed to be the most common musculoskeletal soft tissues to be injured. These injuries may be determined by a combination of both intrinsic and extrinsic risk factors. In the last decade, it has been suggested that genetic predisposition contribute to modulating risk of such injuries.[1] Within this category, anterior cruciate ligament (ACL) rupture is one of the most prevalent and severe knee injuries and predominantly occurs during sports participation, primarily via a non-contact mechanism.[2,3] The main function of the ACL is the stabilization of the knee joint and controlling the joint during movement to protect the knee from excessive flexion and hyperextension.[4] ACL injuries severely impair knee kinematics and are therefore a major concern for professional athletes and physically active individuals participating in recreational activities.

Extracellular matrix (ECM) glycoproteins play a crucial role in the development, structure and function of both tendons and ligaments, which are the key elements of the musculoskeletal system. The structural integrity and normal mechanical function of both tendons and ligaments depend on the precise alignment of type I collagen fibrils.[5] For instance, proteoglycans, a distinct subclass of glycoproteins, play a crucial role in regulating collagen fibrillogenesis.[6] ECM glycoproteins also participate in the matrix remodelling and cell signaling pathways facilitating response to mechanical loads thereby modulating the elastic and

* Corresponding author.
E-mail address: Mary-jessica.laguette@uct.ac.za (M.-J.N. Laguette).

https://doi.org/10.1016/j.jsams.2018.10.003
1440-2440/

biomechanical properties of the tendon or ligament.[7,8] Moreover, ECM glycoproteins can modulate cell-matrix interactions, adhesion and migration.[9]

This family includes, among others, tenascin C (TNC) which is encoded by the *TNC* gene located on human chromosome 9 (9q33.1). There is a high level of TNC glycoprotein expression during embryogenesis, as it is involved in organ morphogenesis.[10] However, it is expressed at very low levels in mature organisms, with the exception of disease states, where an increase in TNC expression is observed in response to injury or infection.[11,12] TNC expression is observed in adult tendons and ligaments[13] where it is expressed predominately at the insertions of ligaments as well as in the myotendinous and osteotendinous regions where it is regulated in a dose-dependent manner.[14–16] These regions are particularly important for the transmission of high levels of mechanical force.[17]

Recent studies have highlighted the involvement of several sequence variants within genes encoding macromolecules present in the extracellular matrix of ligaments and tendons.[18–20] Initially, a familial predisposition for ACL injury was uncovered suggesting that patients with an ACL rupture were twice as likely to have a relative with the same ligament damage[22]; studies in twins have also confirmed that assumption.[23] Understanding the causes and molecular mechanisms underpinning ACL injury in conjunction with knowledge of contributing risk factors is pivotal to the prevention of ACL ruptures. Mokone and colleagues first reported an association between a genetic microsatellite marker, a G-T dinucleotide repeat in intron 17, of *TNC* and Achilles tendon injuries in a South African population study.[24]

Saunders et al. further refined and implicated this region by exploring the association of the non-synonymous exonic rs2104772 (T > A) and rs13321 (G > C), as well as intronic rs1330363 (C > T) genetic variants in a combined South African and Australian population.[25] A haplotype containing the C allele of *TNC* rs13321 (G > C), and the A allele of *TNC* rs2104772 (T > A) was significantly associated with the risk of Achilles tendon injuries. Recently, the A allele at rs2104772 was further shown to modulate the risk of rotator cuff injury in combination with other *TNC* variants.[26] However, a Genome Wide Association Study (GWAS) performed using a large cohort from a general American population did not find a genetic association when examining the same *TNC* variants independently, or in combinations, in the context of Achilles tendon injury and ACL injuries.[27]

To date, little is known about the function of these variants, however bioinformatics analysis conducted in previous studies collectively suggest that the region identified may harbour several functional regulatory elements.[25] To expand the current knowledge on the association of inter-individual variations in the *TNC* gene and ACL injury, this study will investigate whether the *TNC* rs1330363 (C > T), rs2104772 (T > A) and rs13321 (G > C) variants, independently or in combinations, contribute to ACL rupture susceptibility in an athletic Polish cohort.

2. Materials and methods

A total of 421 physically active, unrelated, self-reported Caucasian participants were recruited for this case-control genetic association study between the 2009 and 2016. These participants consisted of 229 (164 male) individuals with surgically diagnosed ACL rupture (ACLR) who qualified for ligament reconstruction and 192 (107 male) apparently healthy participants without any history of ACL injuries (CON group). All 229 participants from ACLR group sustained their injury through non-contact mechanisms. The ACLR participants were soccer players (164 males and 65 females) from the Polish 1st, 2nd and 3rd division soccer league (trained 11–14 h per week).

The control group consisted of healthy participants, mainly soccer players (107 males and 85 females), with self-reported no history of ligament or tendon injury. All the male participants (ACLR and CON groups) were from the same soccer teams, of the same ethnicity (as self-reported, all Polish, East-Europeans for ≥3 generations), of similar age (ACLR group = 26 ± 4, control group = 25 ± 3), and had a comparable level of exposure to risk of ACL injury (same volume and intensity of training as well as match play). The ACLR female participants (age 25 ± 4) were soccer players from Polish 1st division soccer league (trained 10–12 h per week) and included amateur skiers. The female participants from CON group (age 29 ± 2) were recruited from sports clubs and wellness centres (physically active for a minimum of 7 h per week).

The procedures followed in the study were conducted ethically according to the principles of the World Medical Association Declaration of Helsinki and ethical standards in sport and exercise science research. This study was approved by the Ethics Committee of the Pomeranian Medical University in Szczecin (approval number 09/KB/IV/2011). All participants were provided with written information on the study including the purpose, procedures, risks, and benefits of participation. After ensuring that the participant had understood the information provided, every participant gave written informed consent. The experimental procedures were conducted in accordance with the set of guiding principles for reporting the results of genetic association studies defined by the Strengthening the Reporting of Genetic Association studies (STREGA) Statement.[28]

The buccal cells donated by the subjects were collected in Resuspension Solution (GenElute Mammalian Genomic DNA Miniprep Kit, Sigma, Germany) with the use of sterile foam-tipped applicators (Puritan, USA). DNA was extracted from the buccal cells using a GenElute Mammalian Genomic DNA Miniprep Kit (Sigma, Germany) according to the manufacturer's protocol. To discriminate *TNC* rs1330363 (C > T), rs2104772 (T > A) and rs13321 (G > C) alleles, a TaqMan Pre-Designed SNP Genotyping Assay (Applied Biosystems) (assay ID: C___8787735_1, C__16182844_10, C__11844394_10 respectively) was used and all samples were genotyped in duplicate on a StepOne Real-Time Polymerase Chain Reaction (RT-PCR) instrument (Applied Biosystems, USA).

Power analysis was performed using QUANTO v1.2.4 (http://hydra.usc.edu/gxe) to calculate sample size for the study. Minor allele frequencies were taken from the 1000 Genomes Project for the EUR population. Briefly, assuming allele frequencies between 0.1 and 0.9 for the 'risk' allele of each variant investigated and a dominant model of inheritance, our current sample size would be adequate to detect an allelic odds ratio (OR) of 1.5 and greater at a power of 80% and a significance level of 5%. The accordance of observed genotypes with the Hardy-Weinberg principle was assessed using the Pearson's Chi-squared test. Genotype and allele frequencies were compared between cases and controls using the Pearson's Chi-squared test and Fisher's exact test using the programming environment R (https://cran.r-project.com, version 3.1.0). To examine the *TNC* genetic interval encompassed by the variants studied, rs1330363 (C > T; intron 15), rs2104772 (T > A; exon 17) and rs13321 (G > C; exon 24), inferred haplotypes were constructed using the genotype data generated together with haplo.stats package (version 1.7.6).[29] This package was further used to compare the frequency distributions of the generated haplotypes between the control and case participants using Haplo.score.[29] Haplo.score is a score statistic based on the strength of the association of a haplotype with a given phenotype. A positive value indicates increased susceptibility to an ACL injury while a negative value indicates reduced susceptibility.[18] The *TNC* inferred haplotype association with ACL injury was analysed in R using the haplo.stats package. Three haplotype effects: additive (an effect depends on the number of copies of a particular haplotype), dom-

Table 1
Genotype and minor allele frequency distributions of the *TNC* rs1330363 (C > T), rs2104772 (T > A) and rs13321 (G > C) variants in all participants as well as male and female participants only between the control (CON) group and anterior cruciate ligament rupture (ACLR) group in a Polish Caucasian cohort.

		All			Male			Female		
		CON	ACLR	p-Value*	CON	ACLR	p-Value#	CON	ACLR	p-Value&
TNC rs1330363 (Intron 15)	*n*	*191*	*228*		*107*	*163*		*84*	*65*	
	TT	86 (45%)	106 (46%)	p = 0.214	50 (47%)	77 (47%)	p = 0.225	36 (43%)	29 (45%)	p = 0.692
	CT	78 (41%)	102 (45%)		40 (37%)	71 (44%)		38 (45%)	31 (48%)	
	CC	27 (14%)	20 (9%)		17 (16%)	15 (9%)		10 (12%)	5 (8%)	
	C allele	132 (35%)	142 (31%)	p = 0.294	74 (35%)	101 (31%)	p = 0.399	58 (35%)	41 (32%)	p = 0.313
	HWE	0.180	0.517		0.086	1		1	0.567	
TNC rs2104772 (Exon 17)	*n*	*190*	*227*		*106*	*162*		*84*	*65*	
	AA	58 (31%)	67 (30%)	p = 0.973	33 (31%)	47 (29%)	p = 0.897	25 (30%)	20 (31%)	p = 0.877
	AT	92 (48%)	111 (49%)		50 (47%)	81 (50%)		42 (50%)	30 (46%)	
	TT	40 (21%)	49 (22%)		23 (22%)	34 (21%)		17 (20%)	15 (23%)	
	T allele	172 (45%)	209 (46%)	p = 0.834	96 (45%)	149 (46%)	p = 0.929	76 (45%)	60 (46%)	p = 0.492
	HWE	0.093	0.806		0.695	1		1	0.619	
TNC rs13321 (Exon 24)	*n*	*191*	*228*		*107*	*163*		*84*	*65*	
	GG	109 (57%)	123 (54%)	p = 0.518	62 (58%)	88 (54%)	p = 0.521	47 (56%)	35 (54%)	p = 0.893
	GC	70 (37%)	84 (37%)		39 (36%)	60 (37%)		31 (37%)	24 (37%)	
	CC	12 (6%)	21 (9%)		6 (6%)	15 (9%)		6 (7%)	6 (9%)	
	C allele	94 (25%)	126 (28%)	p = 0.322	51 (24%)	90 (28%)	p = 0.368	43 (26%)	36 (28%)	p = 0.434
	HWE	0.862	0.233		1	0.328		0.776	0.540	

Genotype and allele frequencies were expressed as the number of participants with the percentage of the total number of participants in parenthesis. The p-values for the exact test of Hardy-Weinberg equilibrium (HWE) for each of the groups are included in the table. Allelic variation reported on the forward orientation of the genome as per convention.

* CON versus ACLR (unadjusted p-values) in All participants.
\# CON versus ACLR (unadjusted p-values) in Male participants.
& CON versus ACLR (unadjusted p-values) in Female participants.

inant (homozygotes and heterozygotes are assumed to have the same effect), recessive (only homozygotes for a particular haplotype have an effect) were examined. The remaining analyses were conducted using STATISTICA version 13.0 (Dell Software, Inc.; Texas, USA).

3. Results

No significant differences in the genotype and allele frequencies were noted when the control and case groups were compared for either of the *TNC* polymorphisms tested (Table 1). Similarly, no significant differences were noted for any of the three polymorphisms tested when only males or females were compared between the control and case groups (Table 1). Genotype and minor allele frequency distributions for each of the polymorphisms, together with the HWE p-values are shown in Table 1.

The *TNC* polymorphisms were found to be in Hardy-Weinberg equilibrium (HWE) for both the control and case groups (Table 1). Variants rs1330363 and rs2104772 were in high linkage disequilibrium (LD), D': 0.969, r2: 0.619, whilst the other combinations were found to be in low LD ($D' \leq 0.717$, r2 ≤ 0.485).

Moreover, inferred haplotype analysis was performed to examine the effect of allelic combinations on the modulation of risk of ACL ruptures. We identified eight inferred haplotypes using the genotype data from the three variants. There were four haplotypes with inferred frequency greater than 5% which accounted for 91.8% of all [rs1330363-rs2104772-rs13321] haplotypes. The most prevalent haplotype was [T; A; G] (41.8%). Inferred haplotype analysis revealed no significant association between the *TNC* gene and predisposition to ACL rupture (Table 2). No significant differences or trends were observed after adjustment for sex (Table 2).

4. Discussion

In the recent years, a number of studies have examined the associations between ACL rupture and various genetic variants, highlighting the importance of genetic predisposition as a risk factor in ACL rupture.[18–21] Due to their biological functions, the genes encoding ECM glycoproteins are promising candidates for modulation of the risk of musculoskeletal soft tissues injuries.

Considering previous reports[18] in the Polish population on proteoglycans, a distinct subgroup of glycoproteins, and studies examining the association of the *TNC* loci in the context of musculoskeletal soft tissue injuries[24–26] as well as the importance of the gene in this specific context, this study examined whether the *TNC* rs1330363 (C > T), rs2104772 (A > T) and rs13321 (G > C) variants were associated with ACL rupture susceptibility in a Polish population. We hypothesised that the current loci in the *TNC* gene would modulate the risk of ACL ruptures in this population, mirroring what was observed in other studies. Interestingly, no significant differences between genotype and allele distributions were observed in our Polish population groups for all loci examined. Likewise, no significant differences or trends were noted when inferred haplotype analyses were performed.

These glycoproteins together with collagens are major components of the extracellular matrix of tendons and ligaments. They play a crucial role in remodelling pathways activated in response to changes in loading and mobilization, during exercise as well as during development.[5] TNC is expressed in dense connective tissues of tendons and ligaments[11,13] in response to mechanical loading[15] and mainly in regions transmitting a high level of mechanical force.[14] The examination of *TNC* gene expression in biopsy samples of chronic Achilles tendinopathies revealed an increase in *TNC* mRNA levels.[30] Genetic association studies conducted in different populations showed that chromosomal region 9q33, where the *TNC* gene is located, may be involved in the predisposition to Achilles tendon injuries. As mentioned, a dinucleotide (GT) microsatellite marker within intron 17 of *TNC* gene was associated with increased susceptibility to Achilles tendon injuries.[24] Allele containing 12 and 14 GT repeats was over-represented in a group of participants with tendon injuries, while the allele containing 13 and 17 GT repeats was under-represented in the same group of participants. Participants with variants of *TNC* gene with 12 and 14 GT repeats had a 6-fold risk of developing Achilles tendon injuries. Saunders and

Table 2
TNC haplotype analysis.

Haplotype	Frequency (%)			Additive			Dominant			Recessive		
[rs1330363; rs2104772; rs13321]	Total	ACLR	CON	Score	p† 0.624*	p‡ 0.639*	Score	p† 0.452*	p‡ 0.497*	Score	p† 0.696*	p‡ 0.845*
[C; T; G]	13.6	12.3	15.1	-1.35	0.176	0.162	-1.76	0.078	0.081	1.15	0.251	0.367
[T; T; G]	17.8	17.6	18.1	0.05	0.961	0.976	-0.14	0.886	0.942	0.51	0.610	0.782
[T; A; G]	41.8	42.1	41.4	0.08	0.932	0.897	0.26	0.798	0.806	-0.15	0.884	0.957
[C; T; C]	18.6	18.8	18.5	0.17	0.866	0.801	-0.10	0.922	0.975	0.74	0.459	0.508

† p-Value not adjusted.
‡ p-Value adjusted for sex.
* Global score statistic p-value; minimum haplotype frequency 5%.

colleagues showed that in a South African and Australian subjects *COL27AI* rs946053, *TNC* rs13321, and *TNC* rs2104772 variants were significantly associated with risk of Achilles tendon injury.[25] The [G;C;A] haplotype of [rs946053;rs13321;rs2104772] variants occurred significantly more frequently in participants suffering from Achilles tendinopathy compared to healthy control. It was suggested that the rs13321 (G > C) locus was within a putative GATA transcription factor binding site, and putative splicing regulatory elements were found 7 and 15 bp downstream.[25] More recently, several variants in the *TNC* gene were associated with degenerative rotator cuff tears.[26] The T allele at rs1138545 (C > T) was shown to be protective in this context. In addition, the combination of the C allele at rs1138545 (C > T), the A allele at rs2104772 (A > T), and the G allele at rs10759752 (A > G) formed the risk-related haplotype [C;A;G]. Of interest, elevated TNC levels have been observed in synovial fluid after injury to human and canine knee joints.[31]

However, our haplotype analysis revealed no association between the *TNC* gene sequence variants and predisposition to ACL rupture, an acute injury, whilst the previous studies implicated risk modulation in chronic injuries such as rotator cuff and Achilles tendon injuries. Interestingly, the results from this study are further mirrored in a recent GWAS investigation involving a large cohort of individuals from the general American population.[27] No significant genetic associations were observed with the same *TNC* SNPS, independently or in combinations, in the context of ACL injury risk. Although, it should be noted that in the GWAS study, several ACL injuries were included ranging from ACL sprains to ACL tears requiring surgery.

Due to the nature of case-control studies, the results of this study need to be confirmed in independent studies with a large sample size. This current study was sufficiently powered to detect associations at the effect size of OR 1.5 and higher. However, it was underpowered to detect small effect sizes of OR less than 1.5. Further independent studies using a larger group of participants are required to replicate or confirm these findings. Future analyses should also consider investigating other variants within the *TNC* gene for a more comprehensive analysis of the risk profiling specific to the Polish population.

5. Conclusion

In conclusion, this is the first study to investigate a potential correlation between the studied *TNC* variants and ACL injury risk in a cohort of athletes. Interestingly, although evidence in the literature implicates *TNC* variants in tendon biology,[24–26] this study and another[27] do not link these loci within *TNC* to ligament injury risk susceptibility. It is likely that the modulation of ACL rupture susceptibility involves numerous genes encoding proteins involved in the structure, development and regeneration of ligaments.[32] This study further supports the theory that there are similarities and differences in the genetic susceptibility of acute and chronic injuries.

Implications

- The *TNC* rs1330363 (C > T), rs2104772 (T > A) and rs13321 (G > C) variants may not modulate susceptibility to ACL injuries.
- Genetic susceptibility to ACL rupture is dependent on the interactions of various gene variants in several genes that encode and/or regulate the proteins that comprise the ligament.
- The interactions of these specific genetic variants in determining risk susceptibility may not contribute to future multifactorial risk models to ACL rupture.

Acknowledgement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. There is no external support for research or funding to report for this manuscript.

References

1. September A, Rahim M, Collins M. Towards an understanding of the genetics of tendinopathy. *Adv Exp Med Biol* 2016; 920:109–116.
2. Gianotti SM, Marshall SW, Hume PA et al. Incidence of anterior cruciate ligament injury and other knee ligament injuries: a national population-based study. *J Sci Med Sport* 2009; 12(6):622–627.
3. Griffin LY, Agel J, Albohm MJ et al. Noncontact anterior cruciate ligament injuries: risk factors and prevention strategies. *J Am Acad Orthop Surg* 2000; 8(3):141–150.
4. MacWilliams BA, Wilson DR, DesJardins JD et al. Hamstrings cocontraction reduces internal rotation, anterior translation, and anterior cruciate ligament load in weight-bearing flexion. *J Orthop Res* 1999; 17(6):817–822.
5. Yoon JH, Halper J. Tendon proteoglycans: biochemistry and function. *J Musculoskelet Neuronal Interact* 2005; 5(1):22–34.
6. Schaefer L, Iozzo RV. Biological functions of the small leucine-rich proteoglycans: from genetics to signal transduction. *J Biol Chem* 2008; 283(31):21305–21309.
7. Jiang C, Shao L, Wang Q et al. Repetitive mechanical stretching modulates transforming growth factor-beta induced collagen synthesis and apoptosis in human patellar tendon fibroblasts. *Biochem Cell Biol* 2012; 90(5):667–674.
8. Yang G, Im HJ, Wang JH. Repetitive mechanical stretching modulates IL-1beta induced COX-2, MMP-1 expression, and PGE2 production in human patellar tendon fibroblasts. *Gene* 2005; 363:166–172.
9. Rosso F, Giordano A, Barbarisi M et al. From cell-ECM interactions to tissue engineering. *J Cell Physiol* 2004; 199(2):174–180.
10. Jones PL, Jones FS. Tenascin-C in development and disease: gene regulation and cell function. *Matrix Biol* 2000; 19(7):581–596.
11. Chiquet-Ehrismann R, Tucker RP. Tenascins and the importance of adhesion modulation. *Cold Spring Harb Perspect Biol* 2011; 3(5).
12. Schellings MW, Pinto YM, Heymans S. Matricellular proteins in the heart: possible role during stress and remodeling. *Cardiovasc Res* 2004; 64(1):24–31.
13. Mackie EJ. Molecules in focus: tenascin-C. *Int J Biochem Cell Biol* 1997; 29(10):1133–1137.
14. Jarvinen TA, Jozsa L, Kannus P et al. Mechanical loading regulates the expression of tenascin-C in the myotendinous junction and tendon but does not induce de novo synthesis in the skeletal muscle. *J Cell Sci* 2003; 116(Pt 5):857–866.
15. Jarvinen TA, Jozsa L, Kannus P et al. Mechanical loading regulates tenascin-C expression in the osteotendinous junction. *J Cell Sci* 1999; 112(Pt 18):3157–3166.
16. Mackie EJ, Ramsey S. Expression of tenascin in joint-associated tissues during development and postnatal growth. *J Anat* 1996; 188(Pt 1):157–165.
17. Chiquet M, Fambrough DM. Chick myotendinous antigen. II. A novel extracellular glycoprotein complex consisting of large disulfide-linked subunits. *J Cell Biol* 1984; 98(6):1937–1946.
18. Cieszczyk P, Willard K, Gronek P et al. Are genes encoding proteoglycans really associated with the risk of anterior cruciate ligament rupture? *Biol Sport* 2017; 34(2):97–103.

19. Stepien-Slodkowska M, Ficek K, Kaczmarczyk M et al. The variants within the COL5A1 gene are associated with reduced risk of anterior cruciate ligament injury in skiers. *J Hum Kinet* 2015; 45:103–111.
20. Rahim M, Gibbon A, Hobbs H et al. The association of genes involved in the angiogenesis-associated signaling pathway with risk of anterior cruciate ligament rupture. *J Orthop Res* 2014; 32(12):1612–1618.
21. Posthumus M, September AV, O'Cuinneagain D et al. The COL5A1 gene is associated with increased risk of anterior cruciate ligament ruptures in female participants. *Am J Sports Med* 2009; 37(11):2234–2240.
22. Flynn RK, Pedersen CL, Birmingham TB et al. The familial predisposition toward tearing the anterior cruciate ligament: a case control study. *Am J Sports Med* 2005; 33(1):23–28.
23. Hewett TE, Lynch TR, Myer GD et al. Multiple risk factors related to familial predisposition to anterior cruciate ligament injury: fraternal twin sisters with anterior cruciate ligament ruptures. *Br J Sports Med* 2010; 44(12):848–855.
24. Mokone GG, Gajjar M, September AV et al. The guanine-thymine dinucleotide repeat polymorphism within the tenascin-C gene is associated with achilles tendon injuries. *Am J Sports Med* 2005; 33(7):1016–1021.
25. Saunders CJ, van der Merwe L, Posthumus M et al. Investigation of variants within the COL27A1 and TNC genes and Achilles tendinopathy in two populations. *J Orthop Res* 2013; 31(4):632–637.
26. Kluger R, Huber KR, Seely PG et al. Novel tenascin-C haplotype modifies the risk for a failure to heal after rotator cuff repair. *Am J Sports Med* 2017; 45(13):2955–2964.
27. Kim SK, Roos TR, Roos AK et al. Genome-wide association screens for Achilles tendon and ACL tears and tendinopathy. *PLoS One* 2017; 12(3):e0170422.
28. Little J, Higgins JP, Ioannidis JP et al. Strengthening the reporting of genetic association studies (STREGA): an extension of the STROBE Statement. *Hum Genet* 2009; 125(2):131–151.
29. Schaid DJ, Rowland CM, Tines DE et al. Score tests for association between traits and haplotypes when linkage phase is ambiguous. *Am J Hum Genet* 2002; 70(2):425–434.
30. Ireland D, Harrall R, Curry V et al. Multiple changes in gene expression in chronic human Achilles tendinopathy. *Matrix Biol* 2001; 20(3):159–169.
31. Chockalingam PS, Glasson SS, Lohmander LS. Tenascin-C levels in synovial fluid are elevated after injury to the human and canine joint and correlate with markers of inflammation and matrix degradation. *Osteoarthritis Cartilage* 2013; 21(2):339–345.
32. Collins M, Raleigh SM. Genetic risk factors for musculoskeletal soft tissue injuries. *Med Sport Sci* 2009; 54:136–149.