Original research

Are *MMP3, MMP8* and *TIMP2* gene variants associated with anterior cruciate ligament rupture susceptibility?

Ewelina Lulińska-Kuklik [a], Masouda Rahim [b], Waldemar Moska [a], Ewelina Maculewicz [c], Mariusz Kaczmarczyk [a], Agnieszka Maciejewska-Skrendo [d], Krzysztof Ficek [e], Pawel Cieszczyk [c,d], Alison V. September [b,*], Marek Sawczuk [a]

[a] *Faculty of Tourism and Recreation, Gdansk University of Physical Education and Sport, Poland*
[b] *Division of Exercise Science and Sports Medicine, Department of Human Biology, Faculty of Health Sciences, University of Cape Town, South Africa*
[c] *Applied Physiology Unit, Military Institute of Hygiene and Epidemiology, Poland*
[d] *Faculty of Physical Education, Gdansk University of Physical Education and Sport, Poland*
[e] *Faculty of Physiotherapy, The Jerzy Kukuczka Academy of Physical Education in Katowice, Poland*

ARTICLE INFO

ABSTRACT

Objectives: Anterior cruciate ligament rupture (ACLR) is a common and severe knee injury which typically occurs as a result of sports participation, primarily via a non-contact mechanism. A number of extrinsic and intrinsic risk factors, including genetics, have been identified thus far. Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteases (TIMPs) play a crucial role in extracellular matrix remodeling of ligaments and therefore the genes encoding MMPs and TIMPs are plausible candidates for investigation with ACL rupture risk.

Design: A case-control genetic association study was conducted on 229 (158 male) individuals with surgically diagnosed primary ACLR, ruptured through non-contact mechanisms and 192 (107 male) apparently healthy participants (CON) without any history of ACLR. All participants were physically active, unrelated, self-reported Caucasians.

Methods: All participants were genotyped for four single nucleotide polymorphisms (SNP): *MMP3* (rs591058C/T, rs679620 G/A), *MMP8* (rs11225395C/T), and *TIMP2* (rs4789932 G/A) using standard PCR assays. Gene-gene interactions were inferred. Single-locus association analysis was conducted using the Chi-square test. SNP-SNP interaction effects were analysed using multifactor dimensionality reduction (MDR) method.

Results: Genotype frequencies did not significantly differ between cases and controls, however, the *MMP3* rs679620 G and rs591058C alleles were significantly overrepresented in cases compared to controls (p = 0.021, OR = 1.38, 95% CI: 1.05–1.81).

Conclusions: These results support the hypothesis that genetic variation within *MMP3* contributes to inter-individual susceptibility to non-contact ACLR. However, these results need to be explored further in larger, independent sample sets.

© 2019 Sports Medicine Australia. Published by Elsevier Ltd. All rights reserved.

Practical implications

- This study further implicates the *MMP3* gene in the susceptibility to soft tissue injuries.

- Although there are no immediate clinical implications, these findings help identify the main biological pathways contributing to injury susceptibility.

- The genetic associations reported may be included in multifactorial risk models to assess an individual's inherent risk of injury.

1. Introduction

Anterior cruciate ligament rupture (ACLR) is a common and severe knee injury with an estimated 250,000 ACLR occurring annually in the United States.[1] ACLR typically occurs as a result

of sports participation, primarily via a non-contact mechanism. Anterior cruciate ligament (ACL) reconstruction remains the gold standard of treatment for those who wish to return to their pre-injury level of function.[1] However, on average, only 65% of patients return to their pre-injury level of sport participation and only 55% return to competitive level sports after surgery.[2] Additional evidence suggests that the rate of return to sport varies with age. Webster and Feller[3] reported the rate of return to sport was significantly higher for athletes 25 years and younger (48% return rate) compared with older athletes aged 26–35 (32% return rate), as well as those over 36 years in age (19% return rate).[3]

Due to the high incidence of injury, high costs for the health care industry, as well as the clinical consequences of ACLR, it is critical we understand the underlying causes and mechanisms. Therefore, an improved understanding regarding the risk factors, aetiology, and mechanisms may aid in screening for ACLRs in the future.[4] Thus far, a number of extrinsic and intrinsic risk factors have been identified,[5] with several genetic risk factors identified to date.[4]

The dense fibrous connective tissue of the ACL is composed of numerous collagen fibres, such as collagens types I, III–VI, XII and XIV, arranged in a hierarchical pattern. Therefore, the genes encoding collagen fibers of the ACL were proposed as potential candidates. Several studies have identified polymorphisms within the *COL1A1*,[6,7] *COL3A1*,[8,9] *COL5A1*[10,11] and *COL12A1*[12] genes to be associated with ACLR. It is also necessary to consider the role proteoglycans (e.g. aggrecan, decorin), glycoproteins (elastin, tenascin C), matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteases (TIMPs) play in maintaining the structural and biological integrity of the ACL. Therefore, one could also consider the genes encoding proteins that are functionally associated with ligaments as potential candidates for investigation.[4]

The MMPs comprise zinc-dependent endopeptidases that belong to a large family of at least 26 structurally-related proteases.[13] These enzymes play a crucial role in remodelling of the extracellular matrix (ECM) by degrading various collagenous and non-collagenous ECM proteins.[13] The human genome encodes four TIMPs that are the natural, endogenous inhibitors of the MMPs and can be secreted or bound to ECM components.[13,14] The TIMPs are crucial to the maintenance of tissue homeostasis as they can inhibit proteinase activity, reverse ECM remodeling, as well as having the ability to directly suppress the proliferation of endothelial cells.

The genes encoding MMPs and TIMPs were previously associated with several musculoskeletal injuries. Posthumus et al.[15] implicated the chromosomal region 11q22, harbouring several MMP genes (*MMP10*, *MMP1*, *MMP3*, *MMP12*) with ACLR susceptibility.[15] Several single nucleotide polymorphisms (SNPs) (rs679620 G/A, rs591058C/T and rs650108 A/G) within *MMP3* were previously associated with risk of chronic Achilles tendinopathy (AT) in two independent study groups from Australia and South Africa.[16,17] Although, there were contrasts between the direction of associations reported by these two studies, the same region was implicated and therefore needs further interrogation. Additionally, the *MMP8* rs11225395C/T SNP was associated with with tendinopathy of the primary posterior tibial tendon[18] and the *TIMP2* rs4789932 G/A polymorphism was significantly associated with Achilles tendon pathology.[19,20]

Based on the collective findings thus far, the genes encoding MMPs and TIMPs remain plausible biological candidates for further investigation of ACLR risk. Therefore, the aim of this case-control genetic association study was to explore the *MMP3* (rs591058C/T, rs679620 G/A), *MMP8* (rs11225395C/T) and *TIMP2* (rs4789932 G/A) genes with susceptibility to ACLRs in a Polish population.

2. Methods

A total of 421 physically active, unrelated, self-reported Caucasian participants were recruited for this case–control genetic association study between the years 2009 and 2016. The participants consisted of 229 (158 male) individuals with surgically diagnosed primary ACLR who qualified for ligament reconstruction (ACLR group) and 192 (107 male) apparently healthy participants without any history of ACLR (CON group). All 229 participants from the ACLR group sustained their injury through non-contact mechanisms.[21]

The ACLR participants were soccer players (158 males and 71 females) from the Polish 1st, 2nd and 3rd division soccer league (trained 11–14 h per week). The control group were healthy, physically-active individuals with the majority playing soccer as their main sport, who 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 (all self-reported Polish, East-Europeans for ≥3 generations), of similar age (ACLR group: 26 ± 4 years and control group: 25 ± 3 years), and had a comparable level of exposure to risk of ACLR (same volume and intensity of training and match play). The ACLR female participants (mean age: 26 ± 6 years) were soccer players from Polish 1st and 2nd division soccer league (trained 10–12 h per week). The female control participants from the CON group (mean age: 29 ± 2 years) were recruited from sports clubs and wellness centers and self-reported as being physically active for a minimum of 7 h per week.

This study was approved by the Ethics Committee of the Pomeranian Medical University in Szczecin (approval number 09/KB/IV/2011). All participants provided written informed consent. This case-control genetic association study is in accordance with the set of guiding principles for reporting the results of genetic association studies defined by the STREGA Statement.[22]

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. All samples were genotyped for the *MMP3* rs591058, rs679620, *MMP8* rs11225395 and *TIMP2* rs4789932 polymorphisms, in duplicate, using TaqMan® Pre-Designed SNP Genotyping Assays (Applied Biosystems) on a StepOne Real-Time Polymerase Chain Reaction (RT-PCR) instrument (Applied Biosystems, USA) following the manufacturer's recommendations.

Assuming minor allele frequencies between 0.2 and 0.5, a sample size of 192 cases would be adequate to detect an allelic odds ratio of 1.7 and greater at a power of 80% and a significance level of 5% (http://biostats.usc.edu/software). Genotype and allele frequencies were compared between cases and controls using Chi-square test (Dell Inc. (2016). Dell Statistica (data analysis software system), version 13, software.dell.com.). Hardy-Weinberg equilibrium (HWE) was tested in R (R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, https://www.R-project.org/.) using the *genetics* package. For analysis of gene-gene interactions, the multifactor dimensionality reduction (MDR) algorithm was applied using the MDR software package (v3.0.2) (http://sourceforge.net).[23] For evaluation of interaction models, we used 10-fold cross-validation, where the dataset was divided into a training set (9/10 of the dataset) and a testing set (1/10 of the dataset). The cross-validation testing score, cross validation consistency (CVC, the number of times the same model was chosen in the training set) were calculated. The statistical significance of MDR models was assessed using permutation testing. P values were obtained from the empirical distribution of cross-validation testing scores.

Table 1
Genotype and allele frequency distributions and p-values of the investigated polymorphisms as well as p-values for the exact tests for Hardy–Weinberg equilibrium (HWE) in the anterior cruciate ligament rupture (ACLR) and control (CON) groups.

SNP		CON (n = 192)	ACLR (n = 229)	P-value	FDR adjusted p-value
	n	192	229		
	TT	30.7% (59)	23.6% (54)	0.072	0.144
	TC	48.4% (93)	46.7% (107)		
MMP3	CC	20.8% (40)	29.7% (68)		
rs591058	T allele	54.9% (211)	46.9% (215)	**0.021**	**0.042**
	C allele	45.1% (173)	53.1% (243)		
	HWE	0.764	0.348		
	n	192	229		
	AA	30.7% (59)	23.6% (54)	0.072	0.144
	AG	48.4% (93)	46.7% (107)		
MMP3	GG	20.8% (40)	29.7% (68)		
rs679620	A allele	54.9% (211)	46.9% (215)	**0.021**	**0.042**
	G allele	45.1% (173)	53.1% (243)		
	HWE	0.764	0.348		
	n	192	228		
	AA	22.4% (43)	17.5% (40)	0.423	0.423
	AG	44.3% (85)	45.2% (103)		
MMP8	GG	33.3% (64)	37.3% (85)		
rs11225395	A allele	44.5% (171)	40.1% (183)	0.198	0.198
	G allele	55.5% (213)	59.9% (273)		
	HWE	0.150	0.365		
	n	192	228		
	CC	30.2% (58)	36.4% (83)	0.229	0.305
	CT	50.5% (97)	49.6% (113)		
TIMP2	TT	19.3% (37)	14.0% (32)		
rs4789932	C allele	55.5% (213)	61.2% (279)	0.094	0.125
	T allele	45.5% (171)	38.8% (177)		
	HWE	0.752	0.512		

Genotype and allele frequencies are expressed as a percentage with the number of participants (n) in parentheses. p-values in bold typeset indicates significance (p < 0.05).

The Bonferroni correction was considered too conservative for this study, as the tests are on the same group of participants and several of the polymorphisms were in tight linkage disequilibrium.[24] Furthermore, there was an *a priori* hypothesis[25] as all four polymorphisms were previously implicated with musculoskeletal soft tissue injury phenotypes in independent populations.[15–20] The FDR (false discovery rate) procedure (Benjamini–Hochberg) was used to adjust for multiple comparisons using the method applied for multiple testing under dependency.[26] FDR was applied separately for the genotypic association and allelic association. Allele frequencies are calculated using the genotype frequencies and as such the null hypotheses of no genotype association and no allelic association are not independent. Therefore, the false discovery rate was controlled separately for genotypes and alleles.

3. Results

All markers conformed to HWE in both the ACLR group and the CON group (Table 1). The *MMP3* rs679620 G/A and rs591058C/T polymorphisms were in complete linkage disequilibrium (LD) (Table 1; D′ = 0.9998, r² = 0.9996). Genotype frequencies were not significantly different between the groups, however, the *MMP3* rs679620 G and rs591058C alleles were significantly over-represented in cases compared to controls (53.1% vs 45.1%, OR = 1.38 [1.05–1.81], p = 0.021, Table 1). As the rs679620 and rs591058 *MMP3* polymorphisms were in complete LD they were not tested as a haplotype. Table 2 presents the results of MDR analysis: the cross-validation consistencies, testing accuracies as well as associated empiric p values generated by 1000 permutation for each number of loci evaluated. We found no interaction between loci. Testing accuracy for all models was close to 50%. The best results (CV consistency of 10, the highest testing accuracy of 51.8% and the lowest prediction error of 48.2%) were provided by a single-locus model including *MMP3*. Permutation testing revealed no statistical significance of any of the MDR models.

Table 2
Results of MDR analysis for ACLR.

Interaction model	CV Consistency	Testing accuracy	P value[a]
MMP3	10	51.8%	0.601
MMP3, TIMP	6	49.9%	0.782
MMP3, MMP8, TIMP	10[b]	50.4%	0.747

CV – cross-validation.
[a] Empirical p value based on 1000 permutations.
[b] CV consistency is 10 for attribute count equal to 3.

4. Discussion

This genetic association study investigated four SNPs in the *MMP3* (rs679620 G/A, rs591058C/T), *MMP8* (rs11225395C/T) and *TIMP2* (rs4789932 G/A) genes with non-contact ACLR risk in a Polish population. The main finding of this study was the over-representation of the *MMP3* rs679620 G and rs591058C alleles in the ACLR group compared to the CON group. No significant differences in genotype frequency distributions were noted nor were any significant gene-gene interactions observed between the loci analyzed.

Previously, the *MMP3* rs679620 SNP was associated with ACLR in a South African study group as part of an inferred haplotype with the *MMP10* rs486055C/T, *MMP1* rs1799750 1G/2G and *MMP12* rs2276109 A/G polymorphisms.[15] Although, no independent associations were noted for the rs679620 SNP, there was a trend for the GG genotype to be over-represented in the controls. Moreover, the rs679620 G allele was consistently observed to be over-represented in the controls when the four-, three- and two-variant haplotypes were inferred. The authors proposed that the low-risk haplotype combinations may be associated with the presence of the G alleles for the *MMP3* rs679620 and *MMP12* rs2276109 variants.[15]

In contrast, our study found the *MMP3* rs679620 G allele and the *MMP3* rs591058C allele may predispose their carriers to ACLR. It is worth noting that all our cases sustained their injury through non-contact mechanisms whereas in the study by Posthumus et al.,[15]

only 41% (54) of the total cases had non-contact ACLRs. As such, the latter study may not have been sufficiently powered to investigate non-contact ACLRs which may have potentially introduced bias in the statistical associations. There is merit in investigating non-contact ACLRs as these injuries are hypothesized to have a greater intrinsic component as injury occurs due to the athlete's own movements[21] and therefore may be more informative in understanding the pathobiology of ACLRs. The discrepancy between the results of the two studies may also be partly explained by the different ethnicities of the two groups: namely, South African Caucasians and Polish East-Europeans. Population stratification is a known confounder for genetic association studies and therefore the differences in genetic associations may be a reflection of the heterogeneity in allele frequencies between the two populations rather than being risk-associated.[27] Consequently, it is essential to investigate genetic risk factors in multiple independent populations to aid in the identification of the main biological pathways underpinning injury risk.

Despite the differing functional properties of tendons and ligaments, the two tissues share several molecular and morphological features. Therefore it is possible to hypothesize that there may be some overlap in the biological mechanisms contributing to the different musculoskeletal soft tissue injury phenotypes. Raleigh et al[16] reported the MMP3 rs679620 GG and rs591058 CC genotypes were independently associated with AT.[16] In addition, El Khoury et al.[20] found the MMP3 rs679620 GG genotype to be overrepresented in participants with Achilles tendon rupture.[20] Gibbon et al.[17] investigated four MMP3 for their individual and collective contribution to ACLRs and AT. Moreover the authors sought to determine any biological similarity between the genetic signatures underpinning acute (ACLR) and chronic (AT) injuries.[17] No associations were observed with risk of ACLR but the 6A allele (rs3025058; −1612 5A/6A) was associated with increased risk of AT. Interestingly, while the 5A-A-T-G (rs3025058 5A/6A, rs679620 G/A, rs591058C/Tand rs650108 G/A) inferred haplotype was associated with decreased risk of AT in the South African group, the opposite 6A-G-C-G haplotype was associated with reduced risk of AT in the Australian group. The authors recommended further interrogation of the MMP3 locus to understand the differences and similarities between acute and chronic injuries.[17]

The research into the genetic contribution of the rs679620 and rs591058 variants is limited. The functional rs679620 variant is a non-synonymous SNP (Glu45Lys) in exon 2, although this polymorphism was not predicted to be damaging (Sorting Intolerant From Tolerant: http://sift.jcvi.org/). This SNP is thought to be in complete LD with the functional rs3025058 (-1612 5A/6A) polymorphism. The 5A allele is reported to have increased transcriptional activity compared to the 6A allele and individuals with the rs3025058 5A allele likely have a rs679620 A allele.[28] The functional relevance of the rs591058C/T polymorphism, situated in intron 4, is yet to be established. However, its potential role as a regulator of gene function that may affect transcription, RNA splicing or stability of translation cannot be excluded. It is also possible that the rs591058 may be in LD with additional polymorphisms on chromosome 11 and form part of a functional haplotypye with outcomes on gene expression or protein function.[15,16]

It is widely accepted that the ECM of ligaments and tendons is dynamic and plays an important role in force transmission and protection from injury.[14] The composition and integrity of the ECM depends on the balance between the degradation of the ECM, which is principally mediated by MMPs, and tissue formation, in other words inhibition of degradation of ECM, which is mediated by TIMPs. Therefore, strict regulation of MMP production and activity is critical to ECM homeostasis.[14] Consequently, disturbances of this equilibrium, both under- and over-production of MMPs,

may lead to disease processes of a fibrotic or degradative nature, respectively.[14]

No significant interactions were noted between the MMP3, MMP8 and TIMP2 genes with ACLR susceptibility despite the known biological interactions between the proteins. MMP3 is a broad-spectrum stromelysin able to cleave collagenous and non-collagenous ECM proteins but unable to cleave the triple-helical fibrillar collagens. MMP3 also contributes to the activation of other proteases, including MMP8.[29] MMP8 is a member of collagenase subfamily capable of degrading triple-helical fibrillar collagens that provide mechanical strength to tissues. Thus affecting the stability and solubility properties of the collagen, and resulting in denaturation of cleavage products that are subsequently degraded by other MMPs, such as MMP2 and MMP9. TIMPs inhibit MMP activity by interacting with the catalytic domain of MMPs and both MMP3 and MMP8 are inhibited by TIMP2.[29] Furthermore, TIMP2 can also indirectly reduce the activity of MMP3 by inhibiting MMP2 which works simulteanously with MMP3 to activate MMP9.

Previous studies have reported associations of polymorphisms within the MMP and TIMP genes with musculoskeletal soft tissue injury phenotypes and therefore these remain important biological candidates for investigation. For example, the MMP3 rs3025058 5A/5A genotype was associated with susceptibility to ACLR in a contact sport group.[30] A study by El Khoury et al.[20] attempted to determine whether the MMP3 rs679620 and TIMP2 rs4789932 SNPs were associated with AT in British Caucasians.[20] The authors reported the TIMP2 rs4789932 polymorphism was significantly associated with Achilles tendon pathology.[19,20] Furthermore, investgation of the rs11225395 (-799C/T) promoter SNP of the MMP8 gene with tendinopathy of the primary posterior tibial tendon (PTT) in a Brazilian population found the TT genotype and T allele to be associated with increased risk of PTT dysfunction.[18]

It is worthy to note that data obtained from the present study should be interpreted with caution. A potential limitation to this genetic association study is relatively small sample size and in particular the lower number of controls to the cases. Therefore this study should be repeated in larger independent study groups. However, a strength of the study was the inclusion of surgically diagnosed primary ACLR that were sustained through non-contact mechanisms as these injuries are considered to have a greater genetic component.

5. Conclusion

In conclusion, this study provides further evidence implicating the MMP3 gene in the susceptibility to ACLRs. Further research is required to explore the roles of the MMP8 and TIMP2 genes in regulating ligament ECM homeostasis during remodeling and contributing to injury risk.

Acknowledgements

References

1. Erickson BJ, Cvetanovich G, Waliullah K et al. Two-stage revision anterior cruciate ligament reconstruction. Orthopedics 2016; 39(3):e456–e464. http://dx.doi.org/10.3928/01477447-20160324-01.

2. Ardern CL, Taylor NF, Feller JA et al. Fifty-five per cent return to competitive sport following anterior cruciate ligament reconstruction surgery: an updated systematic review and meta-analysis including aspects of physical functioning

and contextual factors. *Br J Sports Med* 2014; 48(21):1543–1552. http://dx.doi.org/10.1136/bjsports-2013-093398.

3. Webster KE, Feller JA. Return to level I sports after anterior cruciate ligament reconstruction: evaluation of age, sex, and readiness to return criteria. *Orthop J Sport Med* 2018; 6(8):232596711878804. http://dx.doi.org/10.1177/2325967118788045.

4. Collins M, September AV, Posthumus M. Genetic influences on anterior cruciate ligament injury, in *The anterior cruciate ligament,* 2nd ed., Prodomos Chadwick C, editor, Elsevier, 2018, 8–12. e1.

5. Orchard J, Seward H, McGivern J et al. Intrinsic and extrinsic risk factors for anterior cruciate ligament injury in Australian footballers. *Am J Sports Med* 2001; 29(2):196–200.

6. Khoschnau S, Melhus H, Jacobson A et al. Type I collagen alpha1 Sp1 polymorphism and the risk of cruciate ligament ruptures or shoulder dislocations. *Am J Sports Med* 2008; 36(12):2432–2436. http://dx.doi.org/10.1177/0363546508320805.

7. Ficek K, Cieszczyk P, Kaczmarczyk M et al. Gene variants within the *COL1A1* gene are associated with reduced anterior cruciate ligament injury in professional soccer players. *J Sci Med Sport* 2013; 16(5):396–400. http://dx.doi.org/10.1016/j.jsams.2012.10.004.

8. O'Connell K, Knight H, Ficek K et al. Interactions between collagen gene variants and risk of anterior cruciate ligament rupture. *Eur J Sport Sci* 2015; 15(4):341–350. http://dx.doi.org/10.1080/17461391.2014.936324.

9. Stępień-Słodkowska M, Ficek K, Maciejewska-Karłowska A et al. Overrepresentation of the *COL3A1* AA genotype in Polish skiers with anterior cruciate ligament injury. *Biol Sport* 2014; 32(2):143–147. http://dx.doi.org/10.5604/20831862.1144416.

10. 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. http://dx.doi.org/10.1177/0363546509338266.

11. Stępień-Słodkowska 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(1):103–111. http://dx.doi.org/10.1515/hukin-2015-0011.

12. Posthumus M, September AV, O'Cuinneagain D et al. The association between the *COL12A1* gene and anterior cruciate ligament ruptures. *Br J Sports Med* 2010; 44(16):1160–1165. http://dx.doi.org/10.1136/bjsm.2009.060756.

13. Somerville RPT, Oblander SA, Apte SS. Matrix metalloproteinases: old dogs with new tricks. *Genome Biol* 2003; 4(6):216. http://dx.doi.org/10.1186/gb-2003-4-6-216.

14. Pasternak B, Aspenberg P. Metalloproteinases and their inhibitors-diagnostic and therapeutic opportunities in orthopedics. *Acta Orthop* 2009; 80(6):693–703. http://dx.doi.org/10.3109/17453670903448257.

15. Posthumus M, Collins M, van der Merwe L et al. Matrix metalloproteinase genes on chromosome 11q22 and the risk of anterior cruciate ligament (ACL) rupture. *Scand J Med Sci Sport* 2012; 22(4):523–533. http://dx.doi.org/10.1111/j.1600-0838.2010.01270.x.

16. Raleigh SM, van der Merwe L, Ribbans WJ et al. Variants within the *MMP3* gene are associated with Achilles tendinopathy: possible interaction with the *COL5A1* gene. *Br J Sports Med* 2009; 43(7):514–520. http://dx.doi.org/10.1136/bjsm.2008.053892.

17. Gibbon A, Hobbs H, van der Merwe W et al. The *MMP3* gene in musculoskeletal soft tissue injury risk profiling: a study in two independent sample groups. *J Sports Sci* 2017; 35(7):655–662. http://dx.doi.org/10.1080/02640414.2016.1183806.

18. Godoy-Santos A, Ortiz RT, Junior RM et al. *MMP-8* polymorphism is genetic marker to tendinopathy primary posterior tibial tendon. *Scand J Med Sci Sport* 2014; 24(1):220–223. http://dx.doi.org/10.1111/j.1600-0838.2012.01469.x.

19. El Khoury L, Posthumus M, Collins M et al. Polymorphic variation within the *ADAMTS2ADAMTS14ADAMTS5ADAM12* and *TIMP2* genes and the risk of Achilles tendon pathology: a genetic association study. *J Sci Med Sport* 2013; 16(6):493–498. http://dx.doi.org/10.1016/j.jsams.2013.02.006.

20. El Khoury L, Ribbans WJ, Raleigh SM. *MMP3* and *TIMP2* gene variants as predisposing factors for Achilles tendon pathologies: attempted replication study in a British case-control cohort. *Meta Gene* 2016; 9:52–−55. http://dx.doi.org/10.1016/j.mgene.2016.03.007.

21. 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. http://dx.doi.org/10.1016/j.jsams.2008.07.005.

22. Little J, Higgins JPT, Ioannidis JPA et al. STrengthening the REporting of Genetic Association studies (STREGA) – an extension of the STROBE statement. *Eur J Clin Invest* 2009; 39(4):247–266. http://dx.doi.org/10.1111/j.1365-2362.2009.02125.x.

23. Gui J, Moore JH, Williams SM et al. A simple and computationally efficient approach to multifactor dimensionality reduction analysis of gene-gene interactions for quantitative traits. *PLoS One* 2013; 8(6):e66545. http://dx.doi.org/10.1371/journal.pone.0066545.

24. Nyholt DR. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. *Am J Hum Genet* 2004; 74(4):765–769. http://dx.doi.org/10.1086/383251.

25. Perneger TV. What's wrong with Bonferroni adjustments. *BMJ* 1998; 316(7139):1236–1238.

26. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Ser B* 1995; 57(1):289–300.

27. Wang Y, Localio R, Rebbeck TR. Evaluating bias due to population stratification in epidemiologic studies of gene-gene or gene-environment interactions. *Cancer Epidemiol Biomarkers Prev* 2006; 15(1):124–132. http://dx.doi.org/10.1158/1055-9965.EPI-05-0304.

28. Ye S, Eriksson P, Hamsten A et al. Progression of coronary atherosclerosis is associated with a common genetic variant of the human stromelysin-1 promoter which results in reduced gene expression. *J Biol Chem* 1996; 271(22):13055–13060.

29. Collazos J, Asensi V, Martin G et al. The effect of gender and genetic polymorphisms on matrix metalloprotease (MMP) and tissue inhibitor (TIMP) plasma levels in different infectious and non-infectious conditions. *Clin Exp Immunol* 2015; 182(2):213–219. http://dx.doi.org/10.1111/cei.12686.

30. Malila S, Yuktanandana P, Saowaprut S et al. Association between matrix metalloproteinase-3 polymorphism and anterior cruciate ligament ruptures. *Genet Mol Res* 2011; 10(4):4158–4165. http://dx.doi.org/10.4238/2011.October.31.1.