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Keywords

ACL injury; Gene polymorphism; COL27A1; COL11A1; Polish athlete; COL27A1 rs946053 ; COL11A1 rs3753841

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The *COL27A1* and *COL11A1* gene variants are not associated with the susceptibility to anterior cruciate ligament rupture in Polish athletes

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Keywords: ACL injury, gene polymorphism, *COL27A1*, *COL11A1*, Polish athlete, *COL27A1* rs946053, *COL11A1* rs3753841.

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1. Introduction

Anterior cruciate ligament injuries (ACL-I) are common and debilitating musculoskeletal injuries among athletes, particularly in sports involving movements such as cutting, pivoting, and jumping [1]. These injuries might lead to chronic pain, osteoarthritis, instability, and reduced mobility [2], which can reduce the ability to participate in sports and physical activity and cause depression during the long-term recovery from ACL reconstruction [3]. Understanding the factors that contribute to ACL-I risk and developing effective prevention and treatment strategies has become a crucial area of research in sports medicine.

The pathogenesis of ACL-I is complex and multifactorial. Several biomechanical factors, such as altered landing mechanics [4], muscle weakness [5], and poor neuromuscular control [6], have been identified as significant risk factors for ACL-I. ACL-I risk may also be influenced by environmental factors, such as the playing surface [7] and weather conditions [8]. These elements imply that the training location matters. For example, rubber mats laid on obstacles during an obstacle challenge for army recruits cause ACL tears [9]. Curiously, rye grass is associated with fewer non-contact anterior cruciate ligament injuries than Bermuda grass [10]. Playing indoors and outdoors is also a factor [11]. The incidence of non-contact ACL rupture might also be influenced by sex. Interestingly, a recent systematic review with meta-analysis revealed that female athletes are more prone to ACL injuries compared to male athletes [12], which had also been previously concluded among athletes (adjusted for sport and the level of play) [13]. According to Sutton et al. (2013), an increased quadriceps angle and an increased posterior tibial slope might predispose women to such injuries [14]. Notably, in non-athletes, when age, ACL, and body anthropometric measures were taken into account, it was shown that the female ACL had lower mechanical properties [15]. However, the findings about women are inconsistent with a case-control study reporting that males are more likely than females to sustain an ACL injury (adjusted for age) [16].

Another factor thought to influence ACL rupture is age. A higher risk of ACL damage exists in female youth football players under the age of 14 [17]. Based on a review of insurance data in pediatrics and teenage football players, both boys and girls had an increased incidence of ACL injury claims between the ages of 11 and 12, and the risk continued to rise to age 18 [18]. Of note, non-contact ACL injuries made up 55% of all ACL injuries in adults and 68% of all ACL injuries in adolescents [12].

Training modalities have also been identified as important [19]. For instance, the majority of current models for preventing ACL injuries include several distinct training modalities (core stability/balance, plyometric, and resistance) [20]. How-ever, plyometric workouts are suggested for female athletes to lower their risk of ACL injuries [21]. There are sex and limb differences in hip and knee kinematics and kinetics during anticipated and unanticipated jump landings [21], and thus, current training modalities might ignore key factors within the injury mechanism [21]. Playing rugby and soccer poses a high risk [13, 19].

Furthermore, genetic factors may also play a role in ACL-I susceptibility. There is growing evidence linking gene polymorphisms to ACL-I. For example, variations in genes encoding connective tissue proteins like collagen [22, 23] and elastin [24] are associated with an increased risk of ACL-I. The fibrous connective tissue of tendons and ligaments consists of several collagenous fiber types, i.e., collagen, minor elastic (e.g., elastin), and non-collagenous proteoglycans (e.g., decorin) and glycoproteins (e.g., tenascin) [25].

SNPs can affect mRNA splicing, nucleocytoplasmic export, stability, and translation [26]. The *COL27A1* gene, which is 156 kb long and spans 61 exons, is located on human chromosome 9q32-33 [27] and is involved in the synthesis of type XXVII collagen. The predicted protein product of the human *COL27A1* gene is estimated to consist of 1860 amino acids [28]. *COL27A1* shows robust expression in cartilage during mouse development and maintains its highest abundance in adult cartilage [28]. The rs946053 SNP is predicted to coincide with a binding site for the c-Myc transcription factor. The role of c-Myc in regulating cell growth involves its binding to the regulatory regions of growth-induced genes, functioning as a transcriptional activator [29]. The T-allele in the distal region of the *COL27A1* gene is anticipated to eliminate this binding site, potentially resulting in a decrease in transcriptional repression and consequent abnormal expression of *COL27A1* [30]. Notably, the rs946053 (T/G) polymorphism, along with another tenascin C (*TNC*) variant, has previously been linked to an increased risk of Achilles tendinopathy in research by Saunders et al. [30], who have also proposed that the haplotype rs946053-rs13321-rs210477 might have functional implications on the transcription, structure, and properties of tenascin-C and the alpha-1 chain of type XXVII collagen, thereby warranting

further investigation. Interestingly, the rs1249744 and rs946053 variants of the *COL27A1* gene interact with genes of the extracellular matrix signaling pathways [31]. Recent phylogenetic analysis has also identified the *COL27A1* locus as a candidate locus for bone disorders [28]. *COL27A1* has been identified as a potential gene associated with ACL-I, elbow dysplasia, and hip dysplasia in dogs by Emily et al. [32]. Other polymorphisms of genes encoding collagen proteins most often studied in the context of an increased risk of soft tissue damage are *COL3A1* [33], *COL5A1* [34, 35], *COL11A1*, *COL11A2* [36], *COL12A1* [22], and *COL27A1* [30].

The *COL11A1* gene is involved in the synthesis of collagen, and the highest correlation has been specifically observed for the gene encoding the α 1 chain of collagen XI. This chain is found in cartilage and other connective tissues [37], and the *COL11A1* gene itself has 68 exons and is located on chromosome 1p21 [38]. Emerging evidence suggests a regulatory interplay between collagen types XI and V, which plays a pivotal role in governing fibrillogenesis during tendon development [39]. Lymphocyte enhancer-binding factor 1 (Lef1) mediates the activation of the *COL11A1* promoter through its DNA binding domain, Lef1 [40]. Lef1 has been reported to be required for proper development as well as bone turnover [41] and wound healing [42]. The association between *COL11A1* variants and ACL rupture has not been examined in humans. However, in dogs, *COL11A1* rs8652327(C/T) has been reported to be associated with cranial cruciate ligament rupture [43, 44]. Furthermore, studies have shown that *COL11A1* rs3753841(C/T) is associated with other musculoskeletal soft tissue injuries, which include lumbar disc herniation [45], elbow tendinopathy [46], joint laxity [43], and hip osteoarthritis [47]. These results suggest that *COL11A1* rs3753841(C/T) may be connected to an increased incidence of ACL injury.

To the best of our knowledge, no studies have investigated the association of the *COL27A1* rs1570460 and *COL11A1* rs3753841 variants to ACL injury, respectively, in any population, and the exact mechanisms by which these gene variants increase the risk of ACL-I are not yet fully understood. In addition, our earlier research has indicated that while some genetic variations were connected with ACL damage in Caucasians [48, 49], others were not [50, 51]. Therefore, the pre-sent study aimed to investigate whether the *COL27A1* polymorphism rs94605 and the *COL11A1* rs3753841 polymorphism are independently associated with susceptibility to ACL injury and to assess this hypothesis in the Polish athletic population. This will be the first study to verify this hypothesis in humans.

2. Materials and Methods

2.1. Partcicipants

This study involved 461 unrelated self-reported Caucasian participants be-tween 2009 and 2023, with 233 individuals (161 men and 71 women) comprising the ACL-I case group. The inclusion criteria for this group required a surgical diagnosis of primary ACL-I and eligibility for ligament reconstruction, with injuries sustained without physical contact. The control group (CON) included 228 individuals (143 men and 85 women), who had not previously suffered an ACL injury and were deemed to be in good health. Male members of the ACL-I group were active soccer players in the first, second, and third-level leagues in Poland, with an average training time of 11 to 14 hours per week (mean time: 11.7 ± 1.3) and an average age of 26 years and 8 months (mean age: 26 ± 4 years). Female members of the ACL-I group, with a mean age of 26 ± 6 years, were also soccer players from the Polish first and second-level leagues, with a mean training time of 10 to 12 hours per week (mean time: 11.1 ± 0.6). The male control group consisted of healthy, physically active adults with an average age of 27 years and 1 month (mean age: 26 ± 6 years) and comparable levels of training and competition intensity to the male ACL-I group. The female control subjects were selected from sports clubs and wellness facilities, self-reporting at least 7 hours of physical activity per week (mean time: 9.2 ± 1.4), with an overall mean age of 29 ± 2 years.

Much effort was directed at matching the cases and controls by age, sex, sport modality, and training location to minimize bias. The study and control groups were of similar age,

and the control-to-case ratio was nearly 1:1 in terms of the number of participants. However, it was not possible to match the participants by sex, i.e., the study relied on both females and males. The study was unable to match the participants by either sport modality or training location. However, the level of training time was similar between males and females of the different groups. It was difficult to find the twin pair. All athletes had an individual training problem, depending on the position, the phase of the game, and even the tasks set by the coach before the next match. Table 1 provides a summary of the characteristics of both the ACL cases and controls.

Table 1. Characteristics of the study participants

Group	N (461)	Gender	Age (SD ±$\bar{X}$)	Time of Training (h/week)
ACL	233	Male 161	26 ± 4	11.7 ± 1.3
		Female 71	26 ± 6	11.1 ± 0.6
CON	228	Male 143	26 ± 6	11.2 ± 1.2
		Female 85	26 ± 2	9.2 ± 1.4

2.2. Ethical Approval

The research procedures for this study were approved by the Pomeranian Medical University Ethics Committee in Szczecin, Poland (approval number 09/KB/IV/2011) and the Bioethics Committee for Clinical Research of the Regional Medical Society in Gdansk, Poland (approval number KB 8/16), both of which adhered to the principles of the World Medical Association's Helsinki Declaration. Each participant received a detailed information sheet outlining the study's objectives, procedures, and potential risks and benefits, and they provided informed consent confidentially and anonymously. The study followed the Strengthening the Publishing of Genetic Association Studies (STREGA) Statement [52], which provides guidelines for the reporting of genetic association studies.

2.3. Genetic Analyses

During the post-surgery control, buccal cells were obtained from the study participants using Copan FLOQ Swabs (Copan Diagnostics, Inc., Murrieta, CA, USA). Genomic DNA was isolated from these cells using a GenElute Mammalian Genomic DNA Miniprep Kit (Sigma, Taufkirchen, Germany) according to the manufacturer's protocol. The samples were genotyped twice using an allelic discrimination assay on a StepOne Real-Time Polymerase Chain Reaction (RT-PCR) machine (Applied Biosystems, Bedford, MA, USA) as described by Maculewicz et al. [53]. To discriminate the *COL27A1* rs946053 and *COL11A1* rs3753841 alleles, TaqMan® PreDesigned SNP Genotyping Assays (Applied Biosystems, Waltham, MA, U.S.A.) (assay ID: C___8786223_20, C___2947954_20, respectively). The assays contained primers and fluorescently-labeled (FAM and VIC) probes.

2.4. Statistical Analysis

The statistical analyses were conducted using the R programming environment and R package (version 3.4.0, the R Foundation for Statistical Computing, https://cran.r-project.org). The genotype and allele frequencies were calculated using four inheritance models, i.e., co-dominant, dominant, recessive, and over-dominant, with the aid of the SNPassoc package version 2.1-0. The statistical significance was determined by a p-value threshold of <0.05.

The required sample size for this study was based on previously published research reporting the collagen genotype effects on ACL injuries. A logistic regression model with group assignment (ACL vs CON) as a dependent variable was used. Using the difference between two independent proportions, a two-tailed 10% effect size (5% versus 15% for a frequency of the genotype of interest) at an alpha level of 0.05 and 80% with equal sample sizes yields a sample size of 282 with 141 individuals in each group, suggesting that our study was sufficiently powered.

3. Results

The examined genetic polymorphisms, rs946053 and rs3753841, were found to adhere to the expectations of the Hardy-Weinberg equilibrium (HWE) ($p = 0.924$ and $p = 0.492$, respectively, as demonstrated in Table 2). The minor allele frequencies for the *COL27A1* rs946053 and *COL11A1* rs3753841 polymorphisms are presented in Table 2. Furthermore, the requirements of the Hardy-Weinberg equilibrium were also met when analyzed independently in both the case and the control groups (as shown in Table 3).

Table 2. Minor allele frequencies (MAF) and the Hardy-Weinberg equilibrium (HWE) testing *p*-values of the rs946053 and rs3753841 polymorphisms

SNP	MAF	HWE (*p*-value)	Missing (%)
rs946053	T (56.7%)	0.924	0
rs3753841	A (61.7%)	0.492	0

MAF – minor allele frequency

Table 3. The Hardy-Weinberg equilibrium (HWE) testing by a group

SNP	Control + Cases	Controls	Cases
rs946053	0.924	1.0	1.0
rs3753841	0.492	0.256	0.895

P values of the HWE test separately in the control and cases

These findings suggest that the investigated genetic polymorphisms do not contribute to a predisposition for the phenotype under investigation. Despite thorough analysis, no significant allele-phenotype relationships were detected through allelic association testing for either rs946053 (Chi-square = 3.06, $p = 0.080$, odds ratio (OR) = 1.17, 95% confidence intervals (CI) 0.98-1.65) (Table 4.) or rs3753841 (Chi-square = 1.46, $p = 0.227$, OR = 1.19, 95% CI 0.91-1.55) (Table 5). These results imply that the investigated genetic polymorphisms do not contribute to a predisposition for the phenotype under investigation.

Table 4. Allelic contingency table for the rs946053 polymorphism

Allele (rs946053)	Control	Cases	Total
T	278 (53.2%)	245 (46.8%)	523
G	188 (47.1%)	211 (52.9%)	399
Total	466	456	922

Table 5. Allelic contingency table for the rs3753841 polymorphism

Allele (rs3753841)	Control	Cases	Total
A	297 (63.7%)	272 (59.6%)	569
G	169 (36.3%)	184 (40.4%)	353
Total	466	456	922

The findings of the association analysis assuming various genetic models (modes of inheritance of the supposed risk allele – a minor allele) are reported in Table 6 and Table 7. As in the case of an allelic association, the investigation found no significant associations between genetic polymorphisms and phenotypic status across all models studied, implying that none of the polymorphisms were associated with the risk of non-contact ACL-I under the four models.

Table 6. Analysis of the genetic relationship between the *COL27A1* gene rs946053 G/T polymorphism and non-contact ACL rupture

Model	Genotype	Control (n = 233)	Cases (n = 228)	OR (95% CI)	*p*
Co-dominant	T/T (n=149)	83 (35.6)	66 (28.9)	1	0.193
	G/T (n=225)	112 (48.1)	113 (49.6)	1.27 (0.84-1.92)	
	G/G (n=87)	38 (16.3)	49 (21.5)	1.62 (0.95-2.76)	
Dominant	T/T	83 (35.6)	66 (28.9)	1	0.125
	G/T+G/G	150 (64.4)	162 (71.1)	1.36 (0.92-2.01)	
Recessive	T/T+G/T	195 (83.7)	179 (78.5)	1	0.155
	G/G	38 (16.3)	49 (21.5)	1.40 (0.88-2.25)	
Over-dominant	T/T-G/G	121 (51.9)	115 (50.4)	1	0.749
	G/T	112 (48.1)	113 (49.6)	1.06 (0.74-1.53)	

OR –odds ratio, 95% CI – confidence intervals, *p*-value<0.05 indicates significance

Table 7. Analysis of the genetic relationship between the *COL11A1* gene rs3753841 A/G polymorphism and non-contact ACL rupture

Model	Genotype	Control (n = 233)	Cases (n = 228)	OR (95% CI)	*p*
Co-dominant	A/A (n = 149)	99 (42.5)	80 (35.1)	1	0.249
	G/A (n = 225)	99 (42.5)	112 (49.1)	1.40 (0.94-2.09)	
	G/G (n=87)	35 (15.0)	36 (15.8)	1.27 (0.73-2.21)	
Dominant	A/A	99 (42.5)	80 (35.1)	1	0.103
	G/A+G/G	134 (57.5)	148 (64.9)	1.37 (0.94-1.99)	
Recessive	A/A+G/A	198 (85.0)	192 (84.2)	1	0.819
	G/G	35 (15.0)	36 (15.8)	1.06 (0.64-1.76)	
Over-dominant	A/A-G/G	134 (57.5)	116 (50.9)	1	0.153
	G/A	99 (42.5)	112 (49.1)	1.31 (0.91-1.89)	

OR –odds ratio, 95% CI – confidence intervals, *p*-value<0.05 indicates significance

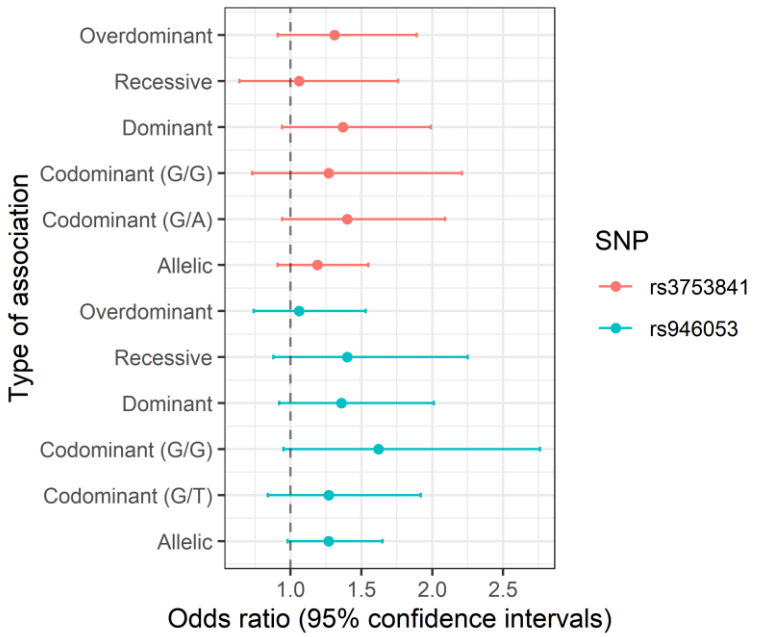

Figure 1. Odds ratios and 95% confidence intervals for the allelic and genotype-based associations

Odds ratios (OR) and 95% confidence intervals for allelic and genotype association analyses are depicted in **Error! Reference source not found.** as a summary of the allelic and genotype-based analyses.

4. Discussion

Following the first longitudinal, prospective cohort study in 1994 [54] that investigated the relationship between cartilage matrix metabolism and knee injury, the focus has shifted toward understanding the genetic factors that may influence ligament rupture and tissue remodeling in the context of ACL injury in different regions. Despite extensive research, our comprehension of the biological processes that lead to musculoskeletal soft tissue injuries remains restricted. Thus, the purpose of this study was to investigate whether the *COL27A1* rs946053, and the *COL11A1* rs3753841 polymorphisms were independently associated with the risk of ACL-I in Polish athletes.

According to the findings of this study, the *COL27A1* gene polymorphisms at 946053 G/T and *COL11A1* rs3753841 A/G are not associated with ACL-I risk in Polish athletes. Additionally, no polymorphisms for either gene appeared to be associated with the risk of non-contact ACL-I in any of the genetic models, including co-dominant, dominant, recessive, and over-dominant. Genotyping results showed no significant differences between healthy and ACL groups in this study.

The *COL27A1* gene, a member of the fibrillar collagen family that encodes the alpha-1 chain of type XXVII collagen, has been identified on chromosome 9q32-33, approximately 708/800 kbp upstream of the *TNC* haplotype [55]. This interesting discovery sheds light on the potential relationship between these genes and their role in musculoskeletal health. It has been shown that this gene plays a role in the later stages of the cartilage modeling phase of endochondral bone formation [56]. Although Saunders et al. [31] found no independent association between gene variants of *TNC* and *COL27A1* with ACL-I and Achilles tendinopathy (AT), their further analysis showed that allelic interactions between sequence variants within *TNC* and the *COL27A1* rs946053 (G/T) variant contributed to AT, particularly with the G-C-A haplotype (rs946053-rs13321-rs2104772) being overrepresented in the AT group of the South African and Australian population [30]. This model of "inter-action between variants" has also been emphasized in the study of Gibbon et al. [55]. However, the investigation yielded no significant differences in the frequency of genotype and allele distributions of the *COL27A1* gene between the control group, the ACL injury group, and the non-ACL injury subgroup among the cohort of individuals with ACL injuries [55]. In addition, recent genome-wide association studies (GWAS) from the UK Biobank cohort have revealed a strong connection between knee pain and the *LOC*105376225 gene, which is located near the *COL27A1* gene, suggesting that *COL27A1* is a promising candidate gene that may contribute to the development of knee lesions [57]. The collective findings of these studies suggest that gene *COL27A1* may pose a potential risk for musculoskeletal injuries, as indicated by the observed interactions between its variants and those of other genes in the development of various conditions.

The rs3753841 polymorphism has been identified as a putative risk factor for soft tissue injuries. Alakhdar [46] was the first to investigate *COL11A1* rs3753841 in relation to elbow tendon pathology (ETP) and discovered that individuals with the CT genotype were more likely to develop ETP. Although the full impact of individual genes on certain conditions remains elusive, the study of gene-gene interactions has emerged as a promising avenue for uncovering novel insights. The T-C-T inferred pseudohaplotype from the combination of *COL11A1* rs3753841 T/C, rs1676486 C/T, and *COL11A2* rs17999079 T/A variants was proposed as a plausible contributor to the development of AT [36]. Individuals carrying the CT genotype of the *COL11A1* rs3753841 gene exhibit an increased susceptibility to elbow tendon pathology (ETP) [46]. Furthermore, the TT genotype of *COL11A1* rs3753841

(C/T) and the T-C inferred haplotype, formed by rs3753841 and rs1676486, are robustly linked to an increased risk of carpal tunnel syndrome (CTS) [58]. The authors indicated that a combination of alleles from these variants could potentially influence the structural or functional properties of the resulting collagen fibril. These findings highlight the potential role of genetic variations in *COL11A1* as contributing factors in the development of soft tissue injuries.

The requirements of the Hardy-Weinberg equilibrium were met when analyzed independently in both the case and the control groups. However, these findings revealed that the genetic variants within *COL27A1* (rs946053) and *COL11A1* (rs3753841) did not exhibit significant dependent associations with ACL-I in Polish athletes, as evidenced by our analysis across multiple statistical models. To date, comprehensive investigations at the individual level regarding the genetic risk conferred by the *COL27A1* gene variant rs946053 (G/T) and *COL11A1* rs3753841 (A/G) in relation to non-contact ACL injury in humans are notably lacking.

Several limitations should be acknowledged when interpreting the results of this research. Initially, we did not account for the potential influence of gender interaction on non-ACL-I, which has been emphasized in our previous work [59]. Multiple studies have consistently indicated that females may be at a higher risk of ACL injury (ACL-I) compared to males [60, 61]. This difference may be attributed to anatomical variations between females and males. However, the precise genetic factors contributing to this disparity remain unclear. Additionally, the inclusion of BMI (Body Mass Index) as a crucial parameter is imperative to mitigate potential bias and enhance the robustness of findings in research pertaining to this subject matter.

The comprehensive understanding of genetic risks in sports injuries necessitates effective interdisciplinary collaboration. ACL-I is a complex condition influenced by multiple factors rather than a single cause; hereditary variables do not always play a significant role in its pathophysiology and related risk. In future genetic studies, it would be both intriguing and essential to meticulously isolate specific variables, including gender, age, BMI, training backgrounds, and injury levels, to gain a more precise understanding of their contributions to ACL-I. Be-sides, new techniques are required to gain a further understanding of the genetic risk associated with ACL-I, such as whole exome sequencing (WES) or genome-wide association studies (GWAS), which have been used to examine interactions be-tween variants or neighboring genes [36, 38].

The paucity of studies exploring the association between the *COL27A1* rs946053 variant and the *COL11A1* rs3753841 variant and ACL injury in any demographic shows a critical knowledge gap. This gap emphasizes the importance of investigating these specific single nucleotide polymorphisms (SNPs) and their potential association with ACL rupture. This study is the first to evaluate *COL27A1* and *COL11A1* polymorphisms in connection to ACL-I. Conducting research in this area presents an opportunity to contribute to the understanding of genetic factors involved in ACL injuries, which could ultimately aid in developing preventative strategies and improving patient outcomes. Further research with larger sample sizes and diverse populations is necessary to validate and extend these preliminary findings.

5. Conclusions

The present study did not establish independent associations between the genotypes of SNPs *COL27A1* rs946053 (G/T) and *COL11A1* rs3753841 (A/G) with non-ACL-I in the Polish cohort. There is abundant room for further progress in determining the potential impact of these two single nucleotide polymorphisms on ACL-I.

References

1. Wetters N, Weber AE, Wuerz TH, Schub DL, Mandelbaum BR. Mechanism of injury and risk factors for anterior cruciate ligament injury. Oper Techn Sport Med. 2016;24:2-6. DOI: 10.1053/j.otsm.2015.09.001
2. Filbay SR, Grindem H. Evidence-based recommendations for the management of anterior cruciate ligament (ACL) rupture. Best Pract & Res Clin Rheumatolog. 2019;33:33-47. DOI: 10.1016/j.berh.2019.01.018
3. Piussi R, Berghdal T, Sundemo D, Grassi A, Zaffagnini S, Sansone M, Samuelsson K, Senorski EH. Self-reported symptoms of depression and anxiety after ACL injury: A systematic review. Orthop J Sports Med. 2022;10:941671549. DOI: 10.1177/23259671211066493
4. Ali N, Robertson DGE, Rouhi G. Sagittal plane body kinematics and kinetics during single-leg landing from increasing vertical heights and horizontal distances: Implications for risk of non-contact ACL injury. Knee. 2014;21:38-46. DOI: 10.1016/j.knee.2012.12.003
5. Wojtys EM, Huston LJ, Schock HJ, Boylan JP, Ashton-Miller JA. Gender differences in muscular protection of the knee in torsion in size-matched athletes. J Bone Joint Surg Am. 2003;85:782-9. DOI: 10.2106/00004623-200305000-00002
6. Muaidi QI, Nicholson LL, Refshauge KM. Do elite athletes exhibit enhanced proprioceptive acuity, range and strength of knee rotation compared with non-athletes? Scand J Med Sci Spor. 2009;19:103-12. DOI: 10.1111/j.1600-0838.2008.00783.x
7. Alentorn-Geli E, Mendiguchia J, Samuelsson K, Musahl V, Karlsson J, Cugat R, Myer GD. Prevention of anterior cruciate ligament injuries in sports—Part I: Systematic review of risk factors in male athletes. Knee Surg Sports Traumatol, Arthrosc. 2014;22:3-15. DOI: 10.1007/s00167-013-2725-3
8. Orchard JW, Powell JW. Risk of knee and ankle sprains under various weather conditions in American football. Med Sci Sport Exer. 2003;35:1118-23. DOI: 10.1249/01.MSS.0000074563.61975.9B
9. Pope RP. Rubber matting on an obstacle course causes anterior cruciate ligament ruptures and its removal eliminates them. Mil Med. 2002;167:355-8. DOI: 10.1093/milmed/167.4.355
10. Orchard JW, Chivers I, Aldous D, Bennell K, Seward H. Rye grass is associated with fewer non-contact anterior cruciate ligament injuries than Bermuda grass. Br J Sports Med. 2005;39:704-9. DOI: 10.1136/bjsm.2004.017756
11. Westin M, Harringe ML, Engström B, Alricsson M, Werner S. Prevention of anterior cruciate ligament injuries in competitive adolescent alpine skiers. Front Sports Act Living. 2020;2:11. DOI: 10.3389/fspor.2020.00011
12. Chia L, De Oliveira Silva D, Whalan M, McKay MJ, Sullivan J, Fuller CW, Pappas E. Non-contact anterior cruciate ligament injury epidemiology in team-ball sports: A systematic review with meta-analysis by sex, age, sport, participation level, and exposure type. Sports Med. 2022;52:2447-67. DOI: 10.1007/s40279-022-01697-w
13. Beynnon BD, Vacek PM, Newell MK, Tourville TW, Smith HC, Shultz SJ, Slauterbeck JR, Johnson RJ. The effects of level of competition, sport, and sex on the incidence of first-time non-contact anterior cruciate ligament injury. Am J Sports Med. 2014;42:1806-12. DOI: 10.1177/0363546514540862
14. Sutton KM, Bullock JM. Anterior cruciate ligament rupture: differences between males and females. J Am Acad Orthop Surg. 2013; 21: 41-50. DOI: 10.5435/JAAOS-21-01-41
15. Chandrashekar N, Mansouri H, Slauterbeck J, Hashemi J. Sex-based differences in the tensile properties of the human anterior cruciate ligament. J Biomech. 2006;39:2943-50. DOI: 10.1016/j.jbiomech.2005.10.031
16. Fernández-Jaén T, López-Alcorocho JM, Rodriguez-Iñigo E, Castellán F, Hernández JC, Guillén-García P. The importance of the intercondylar notch in anterior cruciate ligament tears. Orthop J Sports Med. 2015;3:1806645306. DOI: 10.1177/2325967115597882
17. Hägglund M, Waldén M. Risk factors for acute knee injury in female youth football. Knee Surg Sports Traumatol Arthrosc. 2016;24:737-46. DOI: 10.1007/s00167-015-3922-z
18. Shea KG, Pfeiffer R, Wang JH, Curtin M, Apel PJ. Anterior cruciate ligament injury in pediatric and adolescent soccer players: An analysis of insurance data. J Pediatrn Orthoped. 2004;24:623-8. DOI: 10.1097/00004694-200411000-00005
19. Perini JA, Lopes LR, Guimarães JAM, Goes RA, Pereira LFA, Pereira CG, Mandarino M, Villardi AM, de Sousa EB, Cossich VRA. Influence of type I collagen polymorphisms and risk of anterior cruciate ligament rupture in athletes: A case-control study. BMC Musculoskelet Disord. 2022;23:154. DOI: 10.1186/s12891-022-05105-2

20. Myer GD, Ford KR, PALUMBO OP, Hewett TE. Neuromuscular training improves performance and lower-extremity biomechanics in female athletes. J Strength Cond Res. 2005;19:51-60. DOI: 10.1519/13643.1
21. Brown TN, Palmieri-Smith RM, McLean SG. Sex and limb differences in hip and knee kinematics and kinetics during anticipated and unanticipated jump landings: Implications for anterior cruciate ligament injury. Br J Sports Med. 2009;43:1049-56. DOI: 10.1136/bjsm.2008.055954
22. Posthumus M, September AV, O'Cuinneagain D, van der Merwe W, Schwellnus MP, Collins M. The association between the *COL12A1* gene and anterior cruciate ligament ruptures. Br J Sports Med. 2010;44:1160-5. DOI: 10.1136/bjsm.2009.060756
23. Zhao D, Zhang Q, Lu Q, Hong C, Luo T, Duan Q, Shu S, Lv J, Zhao W. Correlations between the genetic variations in the *COL1A1*, *COL5A1*, *COL12A1*, and *β-fibrinogen* genes and anterior cruciate ligament injury in Chinese patients. J Athl Training. 2020;55:515-21. DOI: 10.4085/1062-6050-335-18
24. El Khoury L, Posthumus M, Collins M, van der Merwe W, Handley C, Cook J, Raleigh SM. *ELN* and *FBN2* gene variants as risk factors for two sports-related musculoskeletal injuries. Int J Sports Med. 2015;36:333-7. DOI: 10.1055/s-0034-1390492
25. Asahara H, Inui M, Lotz MK. Tendons and ligaments: connecting developmental biology to musculoskeletal disease pathogenesis. J Bone Miner Res. 2017;32:1773-82. DOI: 10.1002/jbmr.3199
26. Robert F, Pelletier J. Exploring the impact of single-nucleotide polymorphisms on translation. Front Genet. 2018;9:507. DOI: 10.3389/fgene.2018.00507
27. Gonzaga-Jauregui C, Gamble CN, Yuan B, Penney S, Jhangiani S, Muzny DM, Gibbs RA, Lupski JR, Hecht JT. Mutations in *COL27A1* cause Steel syndrome and suggest a founder mutation effect in the Puerto Rican population. Eur J Hum Genet. 2015;23:342-6. DOI: 10.1038/ejhg.2014.107
28. Pace JM, Corrado M, Missero C, Byers PH. Identification, characterization and expression analysis of a new fibrillar collagen gene, *COL27A1*. Matrix Biol. 2003;22:3-14. DOI: 10.1016/s0945-053x(03)00007-6
29. Halazonetis TD, Kandil AN. Determination of the c-MYC DNA-binding site. Proc Natl Acad Sci U S A. 1991;88:6162-6. DOI: 10.1073/pnas.88.14.6162
30. Saunders CJ, van der Merwe L, Posthumus M, Cook J, Handley CJ, Collins M, September AV. Investigation of variants within the *COL27A1* and *TNC* genes and Achilles tendinopathy in two populations. J Orthop Res. 2013;31:632-7. DOI: 10.1002/jor.22278
31. Saunders CJ, van der Merwe L, Cook J, Handley CJ, Collins M, September AV. Extracellular matrix proteins interact with cell-signaling pathways in modifying risk of Achilles tendinopathy. J Orthop Res. 2015;33:898-903. DOI: 10.1002/jor.22820
32. Binversie EE, Momen M, Rosa GJ, Davis BW, Muir P. Across-breed genetic investigation of canine hip dysplasia, elbow dysplasia, and anterior cruciate ligament rupture using whole-genome sequencing. Front Genet. 2022;13. DOI: 10.3389/fgene.2022.913354
33. O'Connell K, Knight H, Ficek K, Leonska-Duniec A, Maciejewska-Karlowska A, Sawczuk M, Stepien-Slodkowska M, O'Cuinneagain D, van der Merwe W, Posthumus M. Interactions between collagen gene variants and risk of anterior cruciate ligament rupture. Eur J Sport Sci. 2015;15:341-50. DOI: 10.1080/17461391.2014.936324
34. Altinisik J, Meric G, Erduran M, Ates O, Ulusal AE, Akseki D. The BstUI and DpnII variants of the *COL5A1* gene are associated with tennis elbow. Am J Sports Med. 2015;43:1784-9. DOI: 10.1177/0363546515578661
35. Posthumus M, Schwellnus MP, Collins M. The *COL5A1* gene: A novel marker of endurance running performance. Med Sci Sports Exerc. 2011;43:584-9. DOI: 10.1249/MSS.0b013e3181f34f4d
36. Hay M, Patricios J, Collins R, Branfield A, Cook J, Handley CJ, September AV, Posthumus M, Collins M. Association of type XI collagen genes with chronic Achilles tendinopathy in independent populations from South Africa and Australia. Br J Sports Med. 2013;47:569-74. DOI: 10.1136/bjsports-2013-092379
37. Tompson SW, Bacino CA, Safina NP, Bober MB, Proud VK, Funari T, Wangler MF, Nevarez L, Ala-Kokko L, Wilcox WR. Fibrochondrogenesis results from mutations in the *COL11A1* type XI collagen gene. Am J Hum Genet. 2010;87:708-12. DOI: 10.1016/j.ajhg.2010.10.009
38. Aswa M, Helmy H, Noweir S, Ismail S, Taha A, Atef A. Impact of rs11024102 *PLEKHA7*, rs3753841 *COL11A1* single nucleotide polymorphisms, and serum levels of oxidative stress markers on the risk of primary angle-closure glaucoma in Egyptians. J Genet Eng Biotechnol. 2022;20:126. DOI: 10.1186/s43141-022-00400-w

39. Wenstrup RJ, Smith SM, Florer JB, Zhang G, Beason DP, Seegmiller RE, Soslowsky LJ, Birk DE. Regulation of collagen fibril nucleation and initial fibril assembly involves coordinate interactions with collagens V and XI in developing tendon. J Genet Eng Biotechnol. 2011;286:20455-65. DOI: 10.1186/s43141-022-00400-w
40. Kleinert R, Prenzel K, Stoecklein N, Alakus H, Bollschweiler E, Hoelscher A, Warnecke-Eberz U. Gene expression of *COL11A1* is a marker not only for pancreas carcinoma but also for adenocarcinoma of the papilla of vater, discriminating between carcinoma and chronic pancreatitis. Anticancer Res. 2015;35:6153-8.
41. van Genderen C, Okamura RM, Farinas I, Quo R, Parslow TG, Bruhn L, Grosschedl R. Development of several organs that require inductive epithelial-mesenchymal interactions is impaired in *LEF*-1-deficient mice. Genes Dev. 1994;8:2691-703. DOI: 10.1101/gad.8.22.2691
42. Phan QM, Fine GM, Salz L, Herrera GG, Wildman B, Driskell IM, Driskell RR. *LEF1* expression in fibroblasts maintains developmental potential in adult skin to regenerate wounds. Elife. 2020;9:e60066. DOI: 10.7554/eLife.60066
43. Beckley S, Dey R, Stinton S, van der Merwe W, Branch T, September AV, Posthumus M, Collins M. The association of variants within types V and XI collagen genes with knee joint laxity measurements. Genes (Basel). 2022;13:2359. DOI: 10.3390/genes13122359
44. Baird AEG, Carter SD, Innes JF, Ollier WE, Short AD. Genetic basis of cranial cruciate ligament rupture (CCLR) in dogs. Connect Tissue Res. 2014;55:275-81. DOI: 10.3109/03008207.2014.910199
45. Mio F, Chiba K, Hirose Y, Kawaguchi Y, Mikami Y, Oya T, Mori M, Kamata M, Matsumoto M, Ozaki K. A functional polymorphism in *COL11A1*, which encodes the α1 chain of type XI collagen, is associated with susceptibility to lumbar disc herniation. Am J Hum Genet. 2007;81:1271-7. DOI: 10.1086/522377
46. Alakhdar Mohmara Y, Cook J, Benítez-Martínez JC, McPeek ER, Aguilar AA, Olivas ES, Hernandez-Sanchez S. Influence of genetic factors in elbow tendon pathology: A case-control study. Sci Rep. 2020;10:1-7. DOI: 10.1038/s41598-020-63030-7
47. Styrkarsdottir U, Lund SH, Thorleifsson G, Zink F, Stefansson OA, Sigurdsson JK, Juliusson K, Bjarnadottir K, Sigurbjornsdottir S, Jonsson S. Meta-analysis of Icelandic and UK data sets identifies missense variants in *SMO*, *IL11*, *COL11A1* and 13 more new loci associated with osteoarthritis. Nat Genet. 2018;50:1681-7. DOI: 10.1038/s41588-018-0247-0
48. Lulinska-Kuklik E, Rahim M, Moska W, Maculewicz E, Kaczmarczyk M, Maciejewska-Skrendo A, Ficek K, Cieszczyk P, September AV, Sawczuk M. Are *MMP3*, *MMP8* and *TIMP2* gene variants associated with anterior cruciate ligament rupture susceptibility? J Sci Med Sport. 2019;22:753-7. DOI: 10.1016/j.jsams.2019.01.014
49. Lulinska-Kuklik E, Leznicka K, Huminska-Lisowska K, Moska W, Michalowska-Sawczyn M, Ossowski Z, Maculewicz E, Cieszczyk P, Kaczmarczyk M, Ratkowski W. The *VEGFA* gene and anterior cruciate ligament rupture risk in the Caucasian population. Biol Sport. 2019;36:3-8. DOI: 10.5114/biolsport.2018.78902
50. Lulinska-Kuklik E, Laguette MN, Moska W, Weber-Rajek M, Ficek K, Puchala R, Cieszczyk P, Sawczuk M, September AV, Maciejewska-Skrendo A. Are TNC gene variants associated with anterior cruciate ligament rupture susceptibility? J Sci Med Sport. 2019;22:408-12. DOI: 10.1016/j.jsams.2018.10.003
51. Sun Z, Cieszczyk P, Lulinska E, Dzitkowska-Zabielska M, Johne M, Huminska-Lisowska K, Michalowska-Sawczyn M, Ficek K, Leonska-Duniec A, Mastalerz A. Are *COL22A1* gene polymorphisms rs11784270 and rs6577958 associated with susceptibility to a non-contact anterior cruciate ligament injury in Polish athletes? Int J Environ Res Public Health. 2023;20:515. DOI: 10.3390/ijerph20010515
52. Little J, Higgins JP, Ioannidis JP, Moher D, Gagnon F, Von Elm E, Khoury MJ, Cohen B, Davey Smith G, Grimshaw J. STrengthening the REporting of Genetic Association Studies (STREGA)—An extension of the STROBE statement. Ann Intern Med. 2009;33:581-98. DOI: 10.7326/0003-4819-150-3-200902030-00011
53. Maculewicz E, Antkowiak B, Antkowiak O, Mastalerz A, Bialek A, Cywinska A, Borecka A, Huminska-Lisowska K, Garbacz A, Lorenz K. *IL-6* polymorphisms are not related to obesity parameters in physically active young men. Genes (Basel). 2021;12:1498. DOI: 10.3390/genes12101498
54. Dahlberg L, Friden T, Roos H, Lark MW, Lohmander LS. A longitudinal study of cartilage matrix metabolism in patients with cruciate ligament rupture—synovial fluid concentrations of aggrecan fragments, stromelysin-1 and tissue inhibitor of metalloproteinase-1. Br J Rheumatol. 1994;33:1107-11. DOI: 10.1093/rheumatology/33.12.1107

55. Gibbon A, Saunders CJ, Collins M, Gamieldien J, September AV. Defining the molecular signatures of Achilles tendinopathy and anterior cruciate ligament ruptures: A whole-exome sequencing approach. PLoS One. 2018;13:e205860. DOI: 10.1371/journal.pone.0205860
56. Hjorten R, Hansen U, Underwood RA, Telfer HE, Fernandes RJ, Krakow D, Sebald E, Wachsmann-Hogiu S, Bruckner P, Jacquet R. Type XXVII collagen at the transition of cartilage to bone during skeletogenesis. Bone. 2007;41:535-42. DOI: 10.1016/j.bone.2007.06.024
57. Meng W, Adams MJ, Palmer CN, Shi J, Auton A, Ryan KA, Jordan JM, Mitchell BD, Jackson RD. Genome-wide association study of knee pain identifies associations with *GDF5* and *COL27A1* in UK Biobank. Commun Biol. 2019;2:321. DOI: 10.1038/s42003-019-0568-2
58. Dada S, Burger MC, Massij F, de Wet H, Collins M. Carpal tunnel syndrome: The role of collagen gene variants. Gene. 2016;587:53-8. DOI: 10.1016/j.gene.2016.04.030
59. Sun Z, Cieszczyk P, Huminska-Lisowska K, Michalowska-Sawczyn M, Yue S. Genetic determinants of the anterior cruciate ligament rupture in sport: An up-to-date systematic review. J Hum Kinet. 2023;88. DOI: 10.5114/jhk/163073
60. Foos MJ, Hickox JR, Mansour PG, Slauterbeck JR, Hardy DM. Expression of matrix metalloprotease and tissue inhibitor of metalloprotease genes in human anterior cruciate ligament. J Orthop Res. 2001;19:642-9. DOI: 10.1016/S0736-0266(00)00071-1
61. Posthumus M, September AV, O Cuinneagain D, van der Merwe W, Schwellnus MP, Collins M. The *COL5A1* gene is associated with increased risk of anterior cruciate ligament ruptures in female participants. Am J Sports Med. 2009;37:2234-40. DOI: 10.1177/0363546509338266

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