GDANSK UNIVERSITY OF PHYSICAL EDUCATION AND SPORT

Zhuo Sun

CORRELATIONS BETWEEN THE GENETIC VARIANTS IN THE *COL22A1*, *COL27A1*, AND *COL11A1* GENE AND NON-CONTACT ANTERIOR CRUCIATE LIGAMENT INJURY IN CAUCASIANS

PHD DISSERTATION

promotor:

prof. dr hab. Paweł Cięszczyk

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Zhuo Sun

KORELACJE MIĘDZY WARIANTAMI GENETYCZNYMI W GENACH *COL22A1*, *COL27A1* I *COL11A1* A BEZKONTAKTOWYM USZKODZENIEM WIĘZĘDŁA KRZYŻOWEGO PRZEDNIEGO W POPULACJI KAUKASKIEJ

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Paper 1

Sun, Z., Cięszczyk, P., Humińska-Lisowska, K., Michalowska-Sawczyn, M., Yue, S. (2023). Genetic Determinants of the Anterior Cruciate Ligament Rupture in Sport: An Up-to-Date Systematic Review. Journal of Human Kinetics.
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Paper 2

Sun, Z., Cięszczyk, P., Lulińska, E., Dzitkowska-Zabielska, M., Johne, M., Humińska-Lisowska, K., Michałowska-Sawczyn, M., Ficek, K., Leońska-Duniec, A., Mastalerz, A., Janczyk, A., & Marek, S. (2022). Are *COL22A1* Gene Polymorphisms rs11784270 and rs6577958 Associated with Susceptibility to a Non-Contact Anterior Cruciate Ligament Injury in Polish Athletes?. International journal of environmental research and public health, 20(1), 515. https://doi.org/10.3390/ijerph20010515
MEiN points value: 140; IF: 4.614

Paper 3

Sun, Z., Bojarczuk, A., & Cieszczyk P. The *COL27A1* and *COL11A1* gene variants are not associated with the susceptibility to anterior cruciate ligament rupture in Polish athletes. Balt J Health Phys Act. 2023;15(3):Article2. https://doi.org/10.29359/BJHPA.15.3.02
MEiN points value: 70; IF: 0.8

2. Introduction

The anterior cruciate ligament (ACL), a crucial stabilizer in the knee, is frequently susceptible to non-contact injuries. ACL injuries, including above all ACL-rapture (ACL-R) are a significant concern in sports medicine, with substantial implications for athletes' performance, physical well-being, and long-term joint health. According to epidemiological data, ACL injuries pose a significant burden in the United States, with a substantial impact on both individuals and healthcare systems. It is estimated that more than 250,000 ACL injuries occur annually in the United States alone [1]. Among these injuries, approximately 65% require surgical intervention, often in the form of reconstructive surgery, followed by an extensive period of rehabilitation to restore function and facilitate return to sports and activities of daily living [2].

The pathophysiology of ACL-R is characterized by its complexity and multiple contributing factors. ACL injury can be subdivided into non-contact and contact injuries based on the underlying causative mechanisms. Non-contact ACL injuries are a prevalent and concerning issue in sports medicine, particularly in activities that involve sudden changes in movement, pivoting, and landing maneuvers [3,4]. Unlike contact ACL injuries, which occur due to direct external force or collision with another player [5], non-contact ACL injuries transpire in the absence of any direct contact or external impact. These injuries often result from intrinsic factors such as biomechanical imbalances, neuromuscular deficiencies [6], and faulty movement patterns [7,8]. The risk of ACL injury (ACL-R) may also be subject to environmental influences, including playing surface [9] and weather conditions [10].

Additionally, gender-related disparities may also contribute to variations in the incidence of non-contact ACL rupture. In a recent systematic review, supported by meta-analysis, it was found that female athletes are more predisposed to ACL injuries than male athletes, which adds an interesting dimension to the research [11]. Sutton et al. [12] reported that elevated quadriceps angle and increased posterior tibial slope could contribute to a heightened predisposition of such injuries in women. Age represents another crucial factor that should be taken into consideration when addressing ACL injuries during exercise. Conducting an extensive systematic review, the data revealed that non-contact ACL injuries constituted 55% of total ACL injuries in adults and 68% in adolescents [13]. These results emphasize a higher vulnerability to ACL injuries in younger age groups, particularly those arising from non-contact incidents. However, there is an absence of randomized controlled trial (RCT) studies investigating the direct relationship between age and ACL injuries. Non-contact ACL injuries represent a significant challenge in sports medicine. Although biomechanical factors are pivotal contributors to the occurrence and risk of ACL injuries, poor neuromuscular control, muscle imbalances, and ligament laxity, playing a crucial role in understanding the underlying mechanisms and developing effective prevention strategies, the influence of genetic factors has gained increasing attention in recent years [14,15].

Understanding the genetic underpinnings of ACL injuries and their interactions with other contextual factors is crucial for developing effective preventive measures,

personalized treatment strategies, and optimizing rehabilitation protocols. Observations conducted by coaches and sports doctors indicated that, certain individuals seem to be predisposed to such injuries more than others under similar risk exposure [16], sparking interest in the potential role of genetics in susceptibility to non-contact ACL injuries. Genome-wide association studies (GWAS) and candidate gene analyses have identified specific genetic markers and variants associated with ACL injury risk. These genetic variations are involved in various biological processes, including collagen synthesis and metabolism, ligament and tendon structure, inflammation, and muscle function. Several genetic variants have been identified as potential contributors to increased ACL injury risk, primarily influencing elements of the body's connective tissue, such as collagen, the building block of ligaments. Genetic polymorphisms in collagen-encoding genes, like *COL1A1*, *COL5A1*, and *COL12A1* have emerged as significant areas of investigation, given their potential impact on ligament structure and resilience [17,18]. For instance, the rs1800012 SNP in *COL1A1* has been investigated in diverse populations to understand its potential influence on ACL injury predisposition [14,19-22]. However, the results of these studies have revealed varying associations between the rs1800012 SNP and ACL injury risk across different major nationalities.

SNPs possess the capacity to modulate mRNA splicing, nucleo-cytoplasmic export, stability, and translation pathways [23]. Collagens are a group of proteins that provide strength, structure, and elasticity to various tissues, including bones, tendons, and articular cartilage [24]. The *COL22A1* gene is located on chromosome 8 in humans and encodes the alpha 1 chain of Collagen Type *XXII*. This collagen type is primarily localized at tissue junctions, with a significant presence at the myotendinous junction (MTJ) [25,26] and support the strengthening of skeletal muscle attachments and the stabilization of MTJ. The primary site of expression for *COL22A1* is in tissues that undergo remodeling or have elastic properties. Studies conducted on zebrafish have revealed that knockdown of the *COL22A1* gene leads to the development of muscular dystrophy due to the disruption of the myotendinous junction [27,28]. According to Miyamoto-Mikami, the T allele of rs6577958 and A allele of 11784270 in the *COL22A1* gene is significantly associated with an increased risk of MTJ in Japanese athletes [25]. However, the precise functions and mechanisms of *COL22A1* in different tissues are still being elucidated. Considering the current database, there exists a paucity of research studies elucidating the correlation between rs6577958 and rs11784270 within the *COL22A1* gene and ACL-R. As such, we have embarked on a primary and critical investigation into the latent risks that might manifest within the Polish cohort.

The *COL27A1* gene, which is 156 kb long and spans 61 exons, is located on human chromosome 9q32-33 [29], and is primarily expressed in tissues undergoing rapid development or growth, such as the growth plates of bones[30], developing cartilage [29], and the connections of tendons [31]. It is involved in regulating the assembly and organization of collagen fibrils in these tissues, contributing to their strength and and flexibility to the musculoskeletal system. There is evidence that abnormal *COL27A1* expression or mutations in the *COL27A1* gene are associated

with a number of connective tissue disorders. A mutation in *COL27A1*, for example, has been linked to Stickler syndrome [32], a connective tissue disorder characterized by abnormalities of the eyes, ears [33], and congenital scoliosis [34]. Additionally, alterations in *COL27A1* expression have been observed in osteoarthritis [35], suggesting its potential involvement in the degenerative processes of articular cartilage. *COL27A1* rs946053 is a specific genetic variant located within the *COL27A1* gene. According to earlier studies, this mutation may be linked to a higher risk of Achilles tendinopathy (AT) in populations from Australia and South Africa [36,37], they have also suggested that the haplotype rs946053-rs13321-rs210477 may carry functional implications for the transcription, structure, and properties of tenascin-C and the alpha-1 chain of type XXVII collagen [36]. The authors postulated that the interplay between genetic variants within genes responsible for collagen fibril components and those involved in extracellular matrix (ECM) signal transduction pathways could provide valuable insights into the underlying mechanisms contributing to the risk of AT. Despite the existing body of research, which is limited in scope, further investigation is imperative to establish a definitive and comprehensive understanding of the intricate relationship between genetic influences and the occurrence of soft tissue injuries.

The *COL11A1* gene itself has 68 exons and is located on chromosome 1p21 [38]. Encoded by the *COL11A1* gene, type *XI* collagen constitutes a pivotal element of the extracellular matrix (ECM) in cartilage tissues. Alongside type II and type IX collagens, type XI collagen contributes to a heterotypic fibril network. This intertwined structure is vital to maintaining the tensile strength of cartilage and enabling it to resist the shear forces exerted on joints during movement [39,40]. Moreover, in the nuanced progression of embryonic development, type XI collagen plays a crucial role in molding and differentiating chondrocytes [41]. Mutations in the genes that encode these collagen types can cause skeletal abnormalities such as chondrodysplasias [42], demonstrating their critical role in cartilage structure and function [43]. To date, there has been no investigation into the potential link between *COL11A1* variants and ACL rupture in human subjects. However, in numerous studies, the single nucleotide polymorphism rs3753841 in the *COL11A1* gene has been consistently associated with primary angle-closure glaucoma (PACG) [44-46]. Within the realm of sports medicine, SNP rs3753841, has been identified as a potential genetic contributor to a variety of musculoskeletal soft tissue injuries. These include AT [47], rotator cuff tendinopathy (RCT) [48], carpal tunnel syndrome [49], OA [50,51] and elbow tendinopathy [52] in humans.

3. Critical research gaps and Research objectives

An emerging body of evidence suggests a compelling link between gene polymorphisms and susceptibility to ACL injuries. Notably, the polymorphism rs1800012 within the *COL1A1* gene has been identified as a potential risk factor for ACL injuries. Gibbon's [19] research revealed a significant over-representation of the rs1800012 TT genotype within the control group compared to the ACL injuries, which robustly supports an association between the rs1800012 polymorphism and ACL

injury risk. However, these results are not entirely consistent across different populations and sports.

Given the underexplored status of SNPs rs11784270 A/C and rs6577958 C/T within the *COL22A1* gene, and the SNPs rs946053 T/G and rs3753841 A/G within the *COL27A1* and *COL11A1* genes respectively, we recognized a significant gap in our understanding of these genetic variants' potential association with ACL injuries. According to the existing data, four SNPs have been implicated in musculoskeletal soft tissue injuries. However, research concerning these SNPs is relatively sparse. Specifically, only a single study has investigated the association of SNPs rs11784270 A/C and rs6577958 C/T with ACL injuries, and this was limited to a Japanese cohort. As for SNPs rs946053 T/G and rs3753841 A/G, no empirical evidence is currently available to test their potential relationship with ACL injuries in humans. The precise contribution of these SNPs to ACL injury susceptibility in athletes is yet to be defined. In response, our study sought to elucidate this association within a cohort of Polish athletes. This study marked the initial endeavor to validate the connection between SNPs rs11784270 A/C and rs6577958 C/T and ACL-R in humans.

This dissertation comprises three papers. Given the comprehensive systematic review conducted by Kaynak et al. six years ago [53], there arises a compelling necessity to enhance and update our comprehension of this field, especially considering the surge in recent research. Consequently, in the first paper of this dissertation, we conducted a meticulous examination of the existing literature to elucidate the role of genetic variants in susceptibility to ACL injuries. This paper serves as a comprehensive synthesis of existing studies on the subject, with an aim to enhance our collective understanding of genetic predisposition to ACL injuries. In Paper 2 and Paper 3, our objective was to explore the potential risk these four SNPs pose for ACL injuries within the Polish population. The underlying hypothesis was that these particular SNPs may contribute to an increased susceptibility to ACL injuries.

Specific objects

Paper 1: This paper is dedicated to the careful examination and synthesis of the latest research findings regarding genetic variants associated with ACL injuries. Our primary objective is to provide a comprehensive and up-to-date overview of this rapidly evolving field.

Paper 2: To analyze the SNPs rs11784270 A/C and rs6577958 C/T within the *COL22A1* gene, postulating their potential contribution to the risk of ACL injuries Polish soccer players.

Paper 3: To investigate whether the *COL27A1* polymorphism rs946053, and *COL11A1* rs3753841 polymorphism are associated independently with susceptibility to ACL injury, and assessed this hypothesis in the Polish athletic population.

4. The summary of the papers included in the dissertation

This dissertation comprises two core experimental papers, both of which utilize a consistent research methodology to examine our central hypothesis, and one systematic review study. The key difference lies in analyzed SNP (*COL 22A1* in paper two, and *COL27A1* and *COL11A1* in paper three) and the sample pool: for Paper 3, we broadened our scope by including samples from new participants. The specific methodology employed in these studies is outlined in detail within the respective publications [54], while the protocol and eligibility criteria for paper 2 is fully described in publication [55].

4.1 Study 1

Given the existing gap in summarizing recent publications on the genetic influence on non-ACL injuries, we conducted Study 1. In this study, we employed the reporting standards of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement, as outlined by Moher et al., 2009, to ensure comprehensive and precise reporting [56]. Additionally, this study has been officially logged in the PROSPERO database (registration number CRD42022368810, registered on 01/11/2022).

To procure high-quality evidence, a meticulous approach was adopted that involved the exploration of five major scientific electronic databases. This rigorous methodology was complemented by stringent eligibility criteria, ensuring the selection of only the most relevant and impactful studies. Additionally, a specific and comprehensive search strategy was employed, facilitating an exhaustive review and thereby enhancing the overall reliability and validity of our findings. The Newcastle-Ottawa Scale (NOS) [57] was employed as a robust tool to evaluate the potential risk of bias within the selected studies (as depicted in Table 1). Following this rigorous assessment, 24 studies were ultimately shortlisted for inclusion in this review, the selection process, clearly illustrated in Figure 1.

Some studies demonstrated no significant difference between the *COL1A1* rs1800012 [19,58] and rs1107946 [59] variants and the risk of ACL ruptures, whereas others suggested a potential protective role of certain SNPs in the same gene. Similarly, conflicting findings were reported for the association of various SNPs in the *COL5A1* [60] and *COL12A1* [61] genes with ACL injury risk. Lulińska and colleagues [62] found that the polymorphisms of MMP10 (C/T rs486055), MMP12 (T/C rs2276109), and MMP1 (-/G rs1799750) did not exhibit significant differences between individuals with ACL rupture and those in the control group.

Table 1. Risk of Bias Assessed by the Newcastle-Ottawa Scale.

Study	Newcastle-Ottawa Scale Score				Design
	Selection	Comparability of case	Expose	Total	
Wanvisa et al., 2019	★★★★	★★	★★	8	Case-control
Lulińska-Kuklik et al., 2019d	★★★★	★	★★	7	Case-control
Lulińska-Kuklik et al., 2020	★★★★	★	★	6	Case-control
Gibbon et al., 2017	★★★★	★	★	6	Case-control
Lulińska-Kuklik et al., 2018	★★★★	★	★★	7	Case-control
Shukla et al., 2020	★★★★	★★	★★	8	Case-control
Daohong et al., 2020	★★★★	★	★	6	Cross-sectional study
Laguette et al., 2020	★★★★	★★	★	7	Case-control
Gibbon et al., 2020	★★★	★	★	5	Case-control
Perini et al., 2022	★★★★	★	★	6	Case-control
Sivertsen et al., 2019	★★	★	★	4	Cohort study
Suijkerbuijk et al., 2019	★★★★	★★	★	7	Case-control
Lulińska-Kuklik et al., 2019b	★★★★	★	★★	7	Case-control
Rahim et al., 2018	★★★★	★★	★	7	Case-control
Shukla et al., 2020	★★★★	★	★★	7	Cross-Sectional study
Lulinska-Kuklik et al., 2019c	★★★★	★	★	6	Case-control
Rahim et al., 2022	★★★★	★	★	6	Case-control
Rahim et al., 2017	★★★★	★	★	6	Case-control
Feldmann et al., 2022	★★★	★	★	5	Case-control
Seale et al., 2020	★★★★	★	★	6	Case-control
Lulińska-Kuklik et al., 2019a	★★★★	★★	★	7	Case-control
Gibbon et al., 2018	★★★★	★★	★	7	Case-control
Willard et al., 2018	★★★★	★★	★	7	Case-control
Cięszczyk et al., 2017	★★★★	★★	★★	8	Case-control

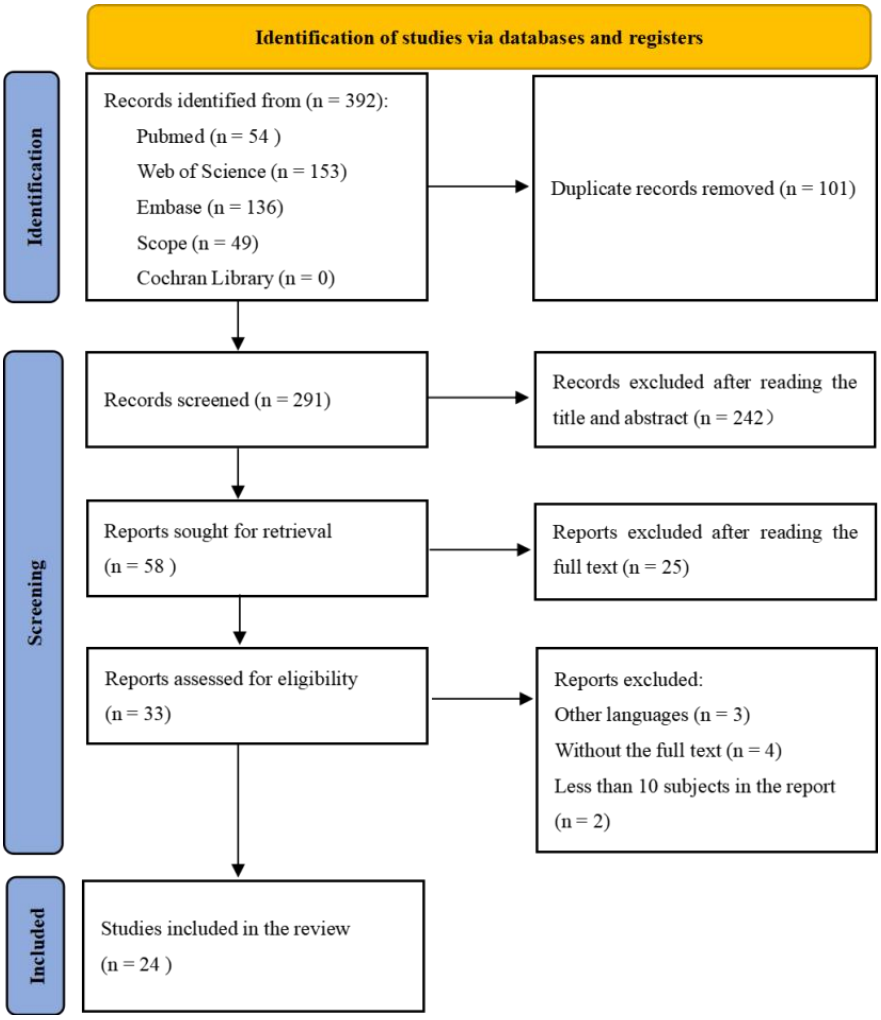

Figure 1. PRISMA flowchart showing the study-selection process.

Key insights from Study 1 illuminate that: a) Even though various investigations have delved into the association between distinct genetic variants and non-contact ACL injuries, the physiological mechanisms underlying these associations remain elusive. b) The potential influence of gender on the relationship between certain gene variants (for instance, BGN rs1042103 and rs1126499) [63], and the risk of ACL injuries was also examined, yielding mixed results. This highlights the necessity to consider gender as a critical variable in such research endeavors. c) Finally, we observed that some studies utilized identical participant pools to scrutinize multiple gene SNP associations [19,64,65]. This methodology may inadvertently introduce bias, underlining the need for a more comprehensive evaluation of polymorphic associations across diverse study populations.

4.2 Study 2

Building upon prior research conducted by our project team, and following meticulous discussions and an extensive literature review, we opted to study SNPs rs11784270 A/C and rs6577958 C/T within the *COL22A1* gene to scrutinize and

clarify any potential genetic risks associated with these SNPs and ACL injuries. We recruited 430 unrelated, self-reported Caucasians between the years 2009 and 2016. Our study comprised 228 individuals in the ACLR case group (157 men and 71 women) who had experienced a surgically diagnosed, non-contact ACL injury. The control group consisted of 202 healthy individuals (117 men and 85 women) with no history of ACL injury. Participants in both groups were physically active and matched for age, sporting exposure, and training intensity. The men primarily played soccer across various league levels in Poland, while the women were drawn from sports clubs and wellness facilities. The buccal cells were collected using Copan FLOQSwabs (Copan Diagnostics, Inc., Murrieta, CA, USA) from all participants. The genotyping was done in duplicate on all samples using a StepOne Real-Time Polymerase Chain Reaction instrument and TaqMan® Pre-Designed SNP Genotyping Assays, specifically for *COL22A1* rs11784270 and *COL22A1* rs6577958. The analysis of our data was conducted utilizing the R programming environment and its associated statistical packages. Table 2 provides a summary of the characteristics of both the ACL cases and controls.

Table 2. Characteristics of the participants in the study 2

	N	Sex	Age	Time of training (h/per week)
Case group: athletes with ACL rapture	228	157 men	26±4	11.9±1.4
		71 women	26±6	11.1±0.6
Control group: athletes without ACL rapture	202	117 men	26±6	11.2±1.2
		85 women	29±2	9.2±1.4

Tables 3 and 4 present the statistical outcomes pertaining to the association between both SNPs within the *COL22A1* gene and ACL injuries. The key revelations of this genetic correlation investigation indicate: (i) the absence of independent associations between rs11784270 A/C and rs6577958 C/T polymorphisms and instances of non-contact ACL ruptures; (ii) none of the examined polymorphisms, whether in co-dominant, dominant, recessive, or over-dominant models, showed any correlation with the risk of non-contact ACL ruptures (Table 3 and Table 4).

Table 3. Analysis of the relationship between non-contact ACL rupture and the *COL22A1* gene's rs11784270 A/C polymorphism.

Model		**CON (n=202)**	**%**	**ACLR (n=228)**	**%**	**OR**	**95% CI**		**p-value**
Codominant	A/A	106	52.5	114	50.0	1.00			
	A/C	69	34.2	95	41.7	1.28	0.85	1.92	0.119
	C/C	27	13.4	19	08.3	0.65	0.34	1.25	
Dominant	A/A	106	52.5	114	50.0	1.00			0.608
	A/C-C/C	96	47.5	114	50.0	1.10	0.76	1.61	
Recessive	A/A-A/C	175	86.6	209	91.7	1.00			0.092
	C/C	27	13.4	19	08.3	0.59	0.32	01.10	
Overdominant	A/A-C/C	133	65.8	133	58.3	1.00			0.109
	A/C	69	34.2	95	41.7	1.38	0.93	02.04	
Allele	A	281	69.6	323	70.8	1.00			0.682
	C	123	31.4	133	29.2	1.06	0,79	1.42	

* ACLR – anterior cruciate ligament rupture, CON – control, OR – odds ratio, 95% CI – confidence intervals.

Table 4. Association analysis of the *COL22A1* gene rs6577958 T/C polymorphism with non-contact ACL rupture.

Model		CON (n=202)	%	ACLR (n=228)	%	OR	95% CI		p-value
Codominant	T/T	131	65.2	150	65.8	1.00			
	C/T	53	26.4	67	29.4	1.10	0.72	1.70	0.284
	C/C	17	08.5	11	04.8	0.57	0.26	1.25	
Dominant	T/T	131	65.2	150	65.8	1.00			0.894
	C/T-C/C	70	34.8	78	34.2	0.97	0.65	1.45	
Recessive	T/T-C/T	184	91.5	217	95.2				0.128
	C/C	17	08.5	11	04.8	0.55	0.25	1.20	
Overdominant	T/T-C/C	148	73.6	161	70.6				0.487
	C/T	53	26.4	67	29.4	1.16	0.76	0.76	
Allele	T	315	78.4	367	80.5	1.00			
	C	87	21.6	89	19.5	1.14	0.82	1.59	0.442

* ACLR – anterior cruciate ligament rupture, CON – control, OR – odds ratio, 95% CI – confidence intervals.

4.3 Study 3

In the third phase of our investigation, we rigorously applied the methodological framework established in our second paper, with a specific objective of exploring the correlation between non-contact ACL injuries and the SNPs *COL27A1* rs946053 and

COL11A1 rs3753841. This particular phase was once again conducted within the context of a Polish cohort, in an ongoing effort to examine potential ethnic genetic variations. The unique distinction in the methodology section between Papers 2 and 3 lies in the recruitment of new participants for Paper 3. Consequently, only this aspect will be elaborated in this section (Table 5).

Table 5. Characteristics of the participants in the study 3.

Group	N (461)	Gender	Age (SD±$\bar{X}$)	Time of Traning (h/week)
ACL	233	Male 161	26 ± 4	11.7 ± 1.3
		Femal 71	26 ± 6	11.1 ± 0.6
CON	228	Male 143	26 ± 6	11.2 ± 1.2
		Femal 85	29 ± 2	9.2 ± 1.4

The outcomes of the Minor Allele Frequencies (MAF) and Hardy-Weinberg equilibrium (HWE) testing P values related to the rs946053 and rs3753841 polymorphisms offer no evidence to suggest that these genetic variations contribute significantly to a predisposition for the phenotype being explored (Table 6). A thorough analysis failed to reveal any noteworthy allele-phenotype correlations via allelic association testing for rs946053 (Chi-square=3.06, P=0.080, Odds Ratio (OR)=1.17, 95% Confidence Intervals (CI) 0.98-1.65) or rs3753841 (Chi-square=1.46, P=0.227, OR=1.19, 95% CI 0.91-1.55). Similar to the allelic association, the study revealed no notable correlations between genetic polymorphisms and the phenotypic status in all models examined. This suggests that none of the studied polymorphisms were linked to an increased risk of non-contact ACL injuries in any of the four models.

Table 6. Allelic contingency table for the and rs946053 and rs3753841.

SNPs	Allele	Control	Cases	Total
rs946053	T	278 (53.2%)	245 (46.8%)	523
	G	188 (47.1%)	211 (52.9%)	399
	Total	466	456	922
rs3753841	A	297 (63.7%)	272 (59.6%)	569
	G	169 (36.3%)	184 (40.4%)	353
	Total	466	456	922

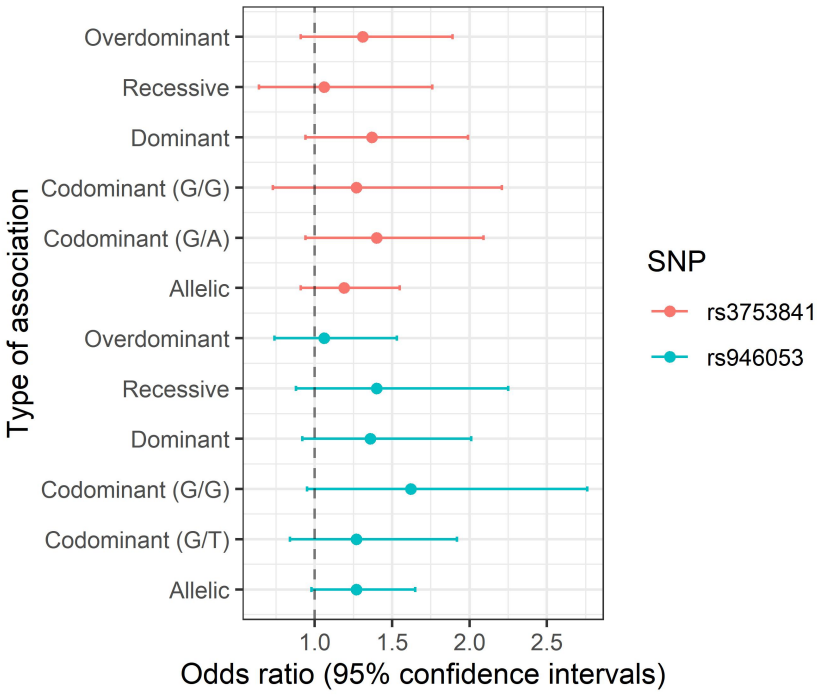

Figure 2. Odds ratios and 95% confidence intervals for the allelic and genotype-based associations.

5. Challenges encountered in the current studies and directions for future research

It is paramount to acknowledge certain limitations while interpreting the findings of this dissertation. In **Paper 1**, owing to the vast scope of genetic variations and the inconsistency of bias risk across different studies, it was unfeasible to carry out a meta-analysis on the existing database.

In light of the simultaneous execution of **Paper 1** and **Paper 2**, several limitations from Paper 2 and Paper 3 were only identified upon completion of Paper 1. For instance, in Paper 2 and Paper 3, the study population comprised of mostly similar subjects. We investigated the association between genetic variants and non-contact ACL injuries across various genes, but the influence of gender was not factored into our research, an aspect that was emphasized in Paper 1. These two pivotal factors amplified the risk of bias in the results.

In retrospect, our papers recommend that future research should strive for global representation to validate a definitive link between genes and ACL injuries, with due consideration given to different nationalities or populations. Furthermore, as some studies have concluded that factors such as gender, BMI, age, and training background may also influence the genetic results, it would be both compelling and critical to diligently isolate these variables. This will facilitate a more nuanced understanding of their individual or combined impact on ACL injury. Such meticulous investigation would serve to generate more specific insights or alternative perspectives on the genetic influence on ACL injury. In addition, the employment of

novel methodologies is crucial to deepen our comprehension of the genetic risks related to ACL injuries in the future.

6. Conclusion

Based on our findings detailed in **Papers 2** and **Paper 3**, there were no discernible relationships between Single Nucleotide Polymorphisms (SNPs) within the genes *COL22A1*, *COL27A1*, and *COL11A1*, and non-contact ACL injuries among Polish athletes. We found there were no significant relationship among all the models. For a more comprehensive understanding of the correlations between genetic variants and ACL ruptures, it is imperative to broaden the research scope, with a specific emphasis on factors such as gender and nationality, as concluded in Paper **1**. Furthermore, to lend greater reliability to these results, they necessitate further substantiation through study in a more expansive and geographically varied sample population.

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Abstract

This dissertation investigated the potential genetic predispositions to non-contact Anterior Cruciate Ligament (ACL) injuries, concentrating on the Single Nucleotide Polymorphisms (SNPs) within the *COL22A1*, *COL27A1*, and *COL11A1* genes among Polish athletes. Comprising three studies, the research illuminated the role of genetic factors in the likelihood of ACL injuries.

The primary study serves as a systematic review, analyzing and compiling recent research findings related to genetic variants implicated in ACL injuries. It aims to bridge the knowledge gap between the current research landscape and previous studies, facilitating the development of a holistic understanding of the rapidly evolving field.

The second Paper and the concluding study (Paper 3) proposed that specific SNPs—rs11784270 and rs6577958 in the *COL22A1* gene, rs946053 in the *COL27A1* gene, and rs3753841 in the *COL11A1* gene—may contribute to an increased risk of non-contact ACL injuries within the Polish athletic population. The findings from these two papers suggest that these genetic markers might be involved in determining the susceptibility to such injuries.

In both Paper 2 and Paper 3, we employed the same methodology but differed in the number of participants, involving 430 and 461 unrelated Caucasians, respectively. These participants were classified into two groups: a case group of individuals with surgically confirmed non-contact ACL injuries, and a control group of healthy individuals with no history of ACL injuries. All participants were physically active and maintained similar levels of sports exposure and training intensity. Buccal cell samples were collected from each participant for genotyping. This genotyping was carried out using a StepOne Real-Time PCR instrument and TaqMan® SNP Genotyping Assays, specifically for *COL22A1* rs11784270 and rs6577958, *COL27A1* rs946053, and rs3753841 in the *COL11A1* gene. Data analysis was conducted utilizing the R programming environment.

In Paper 2 and Paper 3, our efforts to establish a significant dependent relationship between the discussed genetic variants and Anterior Cruciate Ligament (ACL) injuries did not yield conclusive results. We propose that future investigations should be conducted with a broader participant base, which would contribute to the reliability and depth of understanding concerning these relationships. A larger sample size would provide a more robust dataset, ultimately improving the statistical power and enhancing the validity of conclusions drawn. In the course of our investigations, as illustrated across all three studies, we identified gender and population demographics as critical factors in this field of research. These considerations should not be overlooked as they potentially hold significant influence on the results and could pave

the way for insightful comparative studies. Our research, thus far, sets a foundation for subsequent inquiries in this scientific domain. We anticipate that our studies will stimulate further exploration, fostering a more extensive and nuanced understanding of the role of genetic variants in ACL injuries. We hope that our efforts, as preliminary as they may be, will facilitate the emergence of future research that provides more definitive answers in this complex and underexplored area.

Streszczenie

W niniejszej pracy zbadano potencjalne predyspozycje genetyczne do bezkontaktowych urazów więzadła krzyżowego poprzecznego (ACL), koncentrując się na polimorfizmach pojedynczych nukleotydów (SNPs) w genach *COL22A1*, *COL27A1* i *COL11A1* wśród polskich sportowców. Pracę stanowi jednotematyczny cykl trzech publikacji, ujawniający rolę czynników genetycznych w prawdopodobieństwie wystąpienia urazów ACL.

Publlikacja 1 jest przeglądem systematycznym obejmującym analizę i kompilację najnowszych wyników badań dotyczących wariantów genetycznych związanych z urazami ACL. Jego celem jest wypełnienie luki w wiedzy pomiędzy obecnym stanem wiedzy a badaniami poprzednimi, ułatwiając rozwój holistycznego zrozumienia szybko rozwijającej się tej dziedziny.

Publikacja 2 oraz 3 dotyczą specyficznych SNPów – rs11784270 i rs6577958 w genie *COL22A1*, rs946053 *COL27A1* oraz rs3753841 *COL11A1*, które mogą przyczyniać się do zwiększenia ryzyka bezkontaktowych urazów ACL w populacji polskich sportowców. Wyniki tych dwóch prac sugerują, że owe markery genetyczne mogą być zaangażowane w określaniu podatności na takie urazy.

W Publikacji 2 i 3 zastosowano tę samą metodologię, przy czym różnica dotyczyła liczby uczestników (odpowiednio 430 i 461 osoby z populacji kaukaskiej). Uczestnicy zostali podzieleni na dwie grupy: osób z potwierdzonymi chirurgicznie bezkontaktowymi urazami ACL oraz grupę kontrolną obejmującą osoby zdrowe bez historii urazów ACL. Wszyscy uczestnicy byli aktywni fizycznie i mieli podobną regularność treningów. Do genotypowania pobrano komórki z policzka. Genotypowanie przeprowadzono przy użyciu instrumentu PCR StepOne Real-Time oraz aseju TaqMan® SNP, w szczególności w przypadku genów *COL22A1* rs11784270 i rs6577958, *COL27A1* rs946053 oraz rs3753841 w genie *COL11A1*. Analizę danych przeprowadzono przy użyciu programowania R.

Celem Publikacji 2 i 3 było ustalenie związku między omawianymi wariantami genetycznymi a urazami ACL. Badania nie przyniosły jednak jednoznacznych rezultatów. W związku z tym, proponuję, aby przyszłe badania objęły większą liczbę uczestników. Większa liczebność próby zapewniłaby rzetelność zebranych danych i poprawi moc statystyczną. We wszystkich trzech Publikacjach, płeć i demografia ludności zostały określone jako czynniki krytyczne, co stanowi podwaliny do dalszych wnikliwych badań porównawczych. Spodziewa się, że opisane w tezie badania będą stymulować dalsze badania mające na celu zrozumienie roli wariantów genetycznych w urazach ACL, ponieważ ta dziedzina wiedzy pozstaje wciąż niewyczerpana.

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STATEMENT OF THE CO-AUTHORS OF THE PUBLICATION

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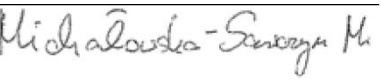
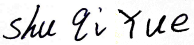
We hereby declare that the individual contribution to the creation of the publication is as follows:

author	Individual (%) contribution	specification	signature
Zhuo Sun	80%	ABCDEF	
Paweł Cięszczyk	10%	AEG	
Kinga Humińska-Lisowska	5%	AD	
Monika Michałowska-Sawczyn	3%	AC	
Shuqi Yue	2%	AB	

* **A** – Study Design, **B** – Data Collection, **C** – Statistical Analysis, **D** – Data Interpretation, **E** – Manuscript Preparation, **F** – Literature Search, **G** – Funds Collection

signature of the candidate for the doctoral degree  promoter's signature 

START for Ph.D.
in SPORT & FITNESS SCIENCES

STATEMENT OF THE CO-AUTHORS OF THE PUBLICATION

Sun, Z., Cięszczyk, P., Luli ń ska, E., Dzitkowska-Zabielska, M., Johne, M., Humi ń ska-Lisowska, K., Michałowska-Sawczyn, M., Ficek, K., Leońska-Duniec, A., Mastalerz, A., Janczyk, A., & Marek, S. (2022). *Are COL22A1 Gene Polymorphisms rs11784270 and rs6577958 Associated with Susceptibility to a Non-Contact Anterior Cruciate Ligament Injury in Polish Athletes?. International journal of environmental research and public health, 20(1),* 515. https://doi.org/10.3390/ijerph20010515

We hereby declare that the individual contribution to the creation of the publication is as follows:

author	Individual (%) contribution	specification	signature
Zhuo Sun	65%	ACDEF	
Paweł Cięszczyk	5%	AG	
Ewelina Lulińska	3%	BD	
Magdalena Dzitkowska-Zabielska	3%	AE	
Monika Johne	3%	CD	
Kinga Humińska-Lisowska	3%	A	
Monika Michałowska-Sawczyn	3%	A	
Krzysztof Ficek	3%	G	
Andrzej Mastalerz	3%	CD	
Agata Leońska-Duniec	3%	CD	
Arkadiusz Janczyk	3%	D	
Sawczuk Marek	3%	E	

* **A** – Study Design, **B** – Data Collection, **C** – Statistical Analysis, **D** – Data Interpretation, **E** – Manuscript Preparation, **F** – Literature Search, **G** – Funds Collection

signature of the candidate for the doctoral degree promoter's signature

START for Ph.D.
in SPORT & FITNESS SCIENCES

STATEMENT OF THE CO-AUTHORS OF THE PUBLICATION

Sun, Z., Bojarczuk , A., & Cieszczyk , P. The COL27A1 and COL11A1 gene variants are not associate d with thesusceptibility to anterior cruciate ligament rupture in Polish athletes. Balt J Health Phys Act. 20 23;15(3):Article2. https://doi.org/10.29359/BJHPA.15.3.02

We hereby declare that the individual contribution to the creation of the publication is as follows:

author	Individual (%) contribution	specification	signature
Zhuo Sun	65%	ADEF	Zhuo Sun
Aleksandra Bojarczuk	20%	DEF	Aleksandra Bojarczuk
Paweł Cięszczyk	15%	ABCG	Paweł Cięszczyk

* **A** – Study Design, **B** – Data Collection, **C** – Statistical Analysis, **D** – Data Interpretation, **E** – Manuscript Preparation, **F** – Literature Search, **G** – Funds Collection

signature of the candidate for the doctoral degree Zhuo Sun promoter's signature Paweł Cięszczyk

ZHUO SUN

♦ +48-785759498 ♦ zhuo.sun@awf.gda.pl Gdansk, Poland

RESEARCH INTEREST

Sport genetic. My current research is focused on the anterior cruciate ligament injury, sport and genotype.

Education

Year	Institution	Statues
02/ 2021- Current	Gdansk University of Physical Education and Sport	Ph.D. Candidate
09/2016 – 6/2019	University of Shanxi	M.S.(Sports Physiology and Anatomy)
09/2012– 07/2016	University of Dalian	B.S. (Kinesiology)

Currently, I am taking academic lectures at Gdansk University of Physical Education and Sport. I am planning to finish the sixth semester in January 2024. As the schedule shows below:

	first year				second year				third year				number of hours	number of hours
	1 sem.		2 sem.		3 sem.		4 sem.		5 sem.		6 sem.			
	hours	ECTS	hours	ECTS	hours	ECTS	hours	ECTS	hours	ECTS	hours	ECTS		
Current Trends in Sport & Fitness Sciences	9	2	9	2	9	2	9	2	9	2	9	2	54	12
Research Ethics	3	1											3	1
Academic Writing			15	3	9	2	6	1	15	3			45	9
Methodology of Research in Sport & Fitness Sciences			15	3	9	2	6	1	15	3			45	9
Data Acquisition, Management & Statistic Analysis			6	1	6	1	6	1	6	1	6	1	30	5
Academic Presentation Skills					6	1	6	1	6	1	3	1	21	4
Management of Reserach Projects					6	1	6	1					12	2
Tutoring & Research Workshop	6	1	6	1	6	1	6	1	6	1	6	1	36	6
Reporting Session & Academic Discussions	3	1	3	1	3	1	3	1	3	1	3	1	18	6
number of hours and ECTS:	21	5	54	11	54	11	48	9	60	12	27	6	**264**	**54**

Professional Experience

03/2021-current **Research**

Gdansk University of Physical Education and Sport, Gdansk, Poland

Division of Molecular Biology

04/2023-07/2023 **Teaching assistant**

Gdansk University of Physical Education and Sport, Gdansk, Poland

Division of Molecular Biology

04/2021-03/2022 **Internship**

Winter Sports Management Center of General Administration of Sport of China, Beijing, China

06/2019 – 09/2019	**Visiting Scholar**
	California State University, LA, U.S.A
	Division of Kinesiology and Nutritional Science
07/2017-06/2019	**Research/ Teaching Assistant**
	University of ShanXi, Taiyuan, China
	Division of Kinesiology
09/2017-10/2017	**Internship**
	Investigator in National Student Fitness Monitoring Program
	University of ShanXi, Taiyuan, China
11/2015-02/2016	**Internship**
	Dalian Sanatorium, Dalian, China

Awards and Scholarship

1. 07/08/2023-20/08/2023 Translator in the international Research Summer Camp, Gdansk, Poland
2. 2022-current Assistant Researcher/Teaching Assistant－Gdansk University of Physical Education and Sport
3. 2019 1st-class Academic Fellowship－University of ShanXi
4. 2018 1st -class Academic Fellowship－University of ShanXi
5. 2017 -2018 Teaching assistantship－University of ShanXi
6. 2017 1st-class Academic Fellowship－University of ShanXi

Research Project/Experience

1. 03/2021-Current－ Genetic factors on the anterior cruciate ligament injury, Gdansk, Poland
   - DNA isolation and DNA sequence genotyping

2. 01/2017-06/2019－Key R & D Projects of Shanxi Province, Taiyuan, China (No. 201803D31030)
   Title: Study on Astaxanthin Against Exercise Oxidation
   - Built the sport fatigue model for rat swimming exercise
   - Collect skeletal muscular samples from rats
   - Collected data, preformed data analysis, and interpreted data
   - Applied H-NMR technology to investigate the effect of increasing burden to skeletal muscular metabolism during treadmill exercise
   - Monitored the change of small molecular metabolites in rats' skeletal muscular during sport fatigue
   - Conducted data analysis and interpreted results independently
   - Wrote manuscripts for peer-review journals

Journal Publications

1. **Sun, Z.**, Cięszczyk, P., Humińska-Lisowska, K., Michalowska-Sawczyn, M., Yue, S. (2023). Genetic Determinants of the Anterior Cruciate Ligament Rupture in Sport: An Up-to-Date Systematic Review. Journal of Human Kinetics. https://doi.org/10.5114/jhk/163073

2. **Sun, Z.,** Cięszczyk, P., Lulińska, E., Dzitkowska-Zabielska, M., Johne, M., Humińska-Lisowska, K., Michałowska-Sawczyn, M., Ficek, K., Leońska-Duniec, A., Mastalerz, A., Janczyk, A., & Marek, S. (2022). Are COL22A1 Gene Polymorphisms rs11784270 and rs6577958 Associated with Susceptibility to a Non-Contact Anterior Cruciate Ligament Injury in Polish Athletes?. International journal of environmental research and public health, 20(1), 515. https://doi.org/10.3390/ijerph20010515

3. **Sun Z**, Bojarczuk A, Cieszczyk P. The COL27A1 and COL11A1 gene variants are not associated with the susceptibility to anterior cruciate ligament rupture in Polish athletes. Balt J Health Phys Act. 2023;15(3):Article2. https://doi.org/10.29359/BJHPA.15.3.02

4. Lei-Lei Wang, **Zhuo Sun**, An-Ping Chen, Li-Jun Wu. ^{1}H-NMR-based metabolomics approach for exploring the effect of astaxanthin supplementation on plasma metabolites after high-intensity physical exercise.J. Mens. Health 2021, 17(3), 122– 131.

5. Wang L-l, Chen A-p, Li J-y, **Sun Z**, Yan S-l, Xu K-y. Mechanism of the Effect of High-Intensity Training on Urinary Metabolism in Female Water Polo Players Based on UHPLC- MS Non- Targeted Metabolomics Technique. Healthcare. 2021; 9(4):381.

6. Xinming Guo, Liju Wu, **Zhuo Sun**. Study on Metabolomics of Skeletal Muscle in Rats by Sports Fatigue. *Journal of Tianjin University of Sport*, 2021,36(03):354-359+372.

7. L. Wu, **Z. Sun**, A. Chen, X. Guo, J. Wang. 2019. Effect of astaxanthin and exercise on antioxidant capacity of human body, blood lactic acid and blood uric acid metabolism. *Science & Sports*, 34:348-352.

8. L. Wu, **Z. Sun***, J. Zhao, XM, Guo, J. Wang. 2019. Effect of astaxanthin supplementation on antioxidant capacity, blood lactate and blood uric acid metabolism in human recovery stage after exercise. *Advances in Bioscience and Bioengineering*, 7:60-63.

9. X. Guo, LJ. Wu, **Z. Sun**, J. Zhao. 2019. Effect of astaxanthin supplementation on human metabolomics after acute high-intensity exercise. *Sports Research and Education*, 34:88-96.

10. **Z. Sun**, S. Wang, LJ. Wu. 2018. The enhancement of astaxanthin on exercise metabolism. *Sport*, 10: 151- 152.

11. J. Zhao, **Z. Sun**, LJ. Wu. 2019. The research progress of glutamine supplementation on exercise metabolism. *Bulletin of Sport Science & Technology*, 27: 46-47.

12. LJ. Wu, J. Zhao, **Z. Sun**, YC. Ruan. Oxidative stress and PI3K-Akt pathway during exercise. *Sports Research and Education*, 34: 1-4.

13. Z. Sun. 2017. The mechanism of sudden cardiac death during exercise and the preventive measures. *Bulletin of Sport Science & Technology*, 25: 161- 162.

Conferences

1. **Z. Sun.** 2022. Genetic determinants of injury to ligaments and tendons-systemic review. *In the 3rd international congress of the polish society of muscles, tendons and bonds*. Lodz, Poland

2. **Z. Sun.** 2018. The effect of astaxanthin supplementation on metabonomic in plasma during human quiet states and after acute high-intensity exercise. *In "Combing physical education and medical science proceedings – the fifth sports and health international research seminar"*. Guangzhou, China.

3. **Z. Sun**. 2018. The effect of One-time Exhaustive Swimming on Viscera in High-fat Diet Induced Obesity-prone and Obesity-resistant Rats.In "Combing physical education and medical science proceedings – the fifth sports and health international research seminar". Guangzhou, China.

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Section II – Exercise Physiology and Sports Medicine

Genetic Determinants of the Anterior Cruciate Ligament Rupture in Sport: An Up-to-Date Systematic Review

by
Zhuo Sun [1,], Paweł Cięszczyk [1], Kinga Humińska-Lisowska [1], Monika Michałowska-Sawczyn [1], Shuqi Yue [1]*

Anterior cruciate ligament injuries (ACLIs) are one of the most common knee injuries in sports. Although numerous factors have been related to the risk of ACLIs, it is still unclear why some individuals are more susceptible than others due to the intricate etiology of ACLIs. Several genetic factors have been identified as contributing to ACLIs. This systematic review summarizes the current evidence regarding the genetic causes of ACLIs based on the available literature. Five electronic databases were searched from 2017 to 2022. All titles, abstracts, and full texts were reviewed in detail to determine the inclusions and exclusions. The Newcastle-Ottawa Scale was used to evaluate the risk of bias. The studies' characteristics and results are presented in both narrative and tabular formats. A total of 24 studies examined 31 genes and 62 variants associated with ACLIs in the global population. Ten studies investigated seven collagens and ten SNPs for the ACL injury. The majority of studies found no significant difference in the association of the COL1A1 rs1800012, COL5A1 rs12722, VEGFA rs1570360, IL6R rs2228145, IL6 rs1800795, IL1B rs16944 and rs1143627, however, contrary results were found when nationality and gender were considered together. Conflicting evidence was found for polymorphisms rs2010963, rs699947 of the VEGFA gene in different studies. Due to a lack of data, it was impossible to determine the relationship between the anterior cruciate ligament rupture (ACLR) and the other polymorphisms. More research is required to establish a clear relationship between the ACLR and genetic variants, particularly when gender and nationality are taken into account separately.

***Keywords**: gene polymorphisms; anterior cruciate injury; single-nucleotide polymorphism (SNP)*

Introduction

Anterior cruciate ligament (ACL) injuries are one of the most common knee injuries in sports (Gwiazdon et al., 2019), and more than 70% of ACL injuries occur in a non-contact situation (Jeong, 2021). According to a report, over 250,000 of ACL injuries occur annually in the United States, and approximately 65% of those injuries require reconstructive surgery (Baker, 1998), and a long period of rehabilitation. However, roughly 45% of athletes do not return to competition (Petushek et al., 2019). Considering the increased number, high costs, and detrimental clinical consequences, the understanding of direct causes and mechanisms is needed to decrease the risk of the ACL injury.

An ACL injury is attributed to extrinsic and intrinsic mechanisms. Extrinsic factors are those that can be adjusted to decrease the risk of ACL injury such as the playing surface and exercise intensity. However, although numerous factors have been related to the risk of ACL injury, it is still unclear why some individuals are more susceptible than others due to the intricate aetiology of ACL injuries. Female athletes, for example, have a higher risk of ACL injury than male athletes (Fatahi et al., 2019) in both contact (Montalvo et al., 2019) and non-contact (Larwa et al., 2021) situations, which could be explained by female athletes to have smaller ligament size, a narrower femoral notch, an increased posterior-inferior slope of the lateral tibia plateau, increased knee and generalized laxity, and an increased body mass index (Lin et al., 2018). According to a recent study, ACL injury has a significant hereditary

[1] *Faculty of Physical Education, Gdansk University of Physical Education and Sport, Gdansk, Poland.*
* Correspondence: zhuo.sun@awf.gda.pl

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component, which can reach 69% in families (Magnusson et al., 2020).

In recent years, there has been an increasing amount of evidence supporting the hypothesis that genetic sequence variants play a significant role in the ACL rupture occurrence (Daohong, 2020; Kim et al., 2021), with single nucleotide polymorphisms (SNPs) in the collagen gene already having been linked to genetic susceptibility. Additionally, despite the fact that Kaynak et al. (2017, 2018) and John et al. (2016) highlighted and summarized some DNA polymorphisms, further research is needed to prove that they are directly associated with ACL injuries. Therefore, genetic factors influencing ACL injuries in sports will be updated in this systematic review based on earlier reports.

Methods

Protocol

In this study, the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement was used for reporting (Moher et al., 2009). This systematic review was registered in the PROSPERO database and the registration number is CRD42022368810.

Eligibility Criteria

Studies were considered for inclusion in the systematic review if they satisfied the following criteria: a case-control, cohort, cross-section and randomized controlled experiment that investigated genetic influences on the ACL injury in humans. Studies with previous systematic reviews, animal studies, book chapters, letters, editorials, conference abstracts, and review articles were disregarded. Furthermore, studies not written in English, without full-text were eliminated. Also studies with fewer than ten participants were not taken into consideration.

Search Strategy

The electronic databases PubMed Central, Web of Science, Cochran Library, Embase and Scopus were searched from the 1st of January, 2017 to the 18th of September, 2022 without language restriction, however, only articles in English were taken into consideration. The following search strategy was applied: ('anterior cruciate ligament injury'/exp OR 'anterior cruciate ligament injur*':ti,ab,kw OR 'acl injur*':ti,ab,kw OR 'anterior cruciate ligament tear*':ti,ab,kw OR 'acl tear*':ti,ab,kw OR 'anterior cruciate ligament rupture*':ti,ab,kw OR 'acl rupture*':ti,ab,kw) AND ('heredity'/exp OR 'genetic determinism':ti,ab,kw OR 'genetic effect':ti,ab,kw OR 'genetic factor':ti,ab,kw OR 'genetic phenomena':ti,ab,kw OR 'genetic processes':ti,ab,kw) AND (2018:py OR 20 1 9:py OR 2020:py OR 2021:py OR 2022:py).

Selection Process

Using the aforementioned search strategy, results were searched by the first author, and all of the results were imported into a reference manager (Endnote Vision X9.3.3) to remove duplicates. Two authors independently reviewed the results. Titles and abstracts were used as the initial criteria for selecting suitable studies. If an abstract did not provide sufficient information, full-texts were examined. In the event of discrepancies between two authors, the final decision was made by a third reviewer.

Data Management

Data Items

Data from the included studies were extracted independently by two reviewers. Extracted data items included the author's name, the year of publication, the country or the region, ethnicity of research, the study design, the gene's name, a detailed genotype frequency of cases and controls, authors' definitions of cases and controls, sample size (case and control), sample types, training background of research subjects.

To evaluate the potential risk of bias in case-control, cohort and cross-section studies, the Newcastle-Ottawa Scale (NOS) (Stang, 2010) was used and scored by two reviewers independently. This scale comprises eight questions divided into three categories: research group selection, group comparability, and the ascertainment of either the exposure or the outcome of interest for case-control or cohort studies, respectively. The NOS employs a "star" rating system with a scale from zero to nine stars, and studies with an overall score of 7 were generally considered to have high quality. Each item in the selection and exposure receives one star, with a maximum of two stars provided if it meets the criteria in the comparability section. When there was a disagreement between two reviewers regarding the quality assessment of a study, a third reviewer was invited to participate

in the evaluation process.

Data Synthesis

After analyzing the included studies, it was determined that meta-analysis was not appropriate due to the diversity of genetic variations and the heterogeneity of the risk of bias between investigations. The same genetic variants from multiple studies were compared and evaluated to determine whether genetic variables contributed to the ACLR. As a result, the findings are described in a narrative but systematic review.

Results

Study Selection

From all databases, a total of 392 studies were retrieved. Two authors independently assessed the titles and abstracts of 291 papers after 101 duplicates were eliminated. Next, 242 and then 25 records were excluded after reading the title/abstract and the full text, respectively. Finally, 24 full text studies that met the criteria for inclusion were examined (Figure 1).

Study Characteristics

This systematic review included twenty-four papers published between 2017 and 2022 in English, with summary features presented in Table 1. The total number of participants in the case and control groups was 5377 and 4343, respectively, and they could be classified into Asians, Caucasians, and colored people. Twenty-one case-control studies, two cross-sectional studies, and one cohort study were included. In the Feldmann et al.'s (2022) study, three cohorts were considered: Sweden, Poland, and Australia, while in the Suijkerbuijk et al.'s (2019) study, two cohorts were examined: Sweden and South Africa (European Caucasian ancestry). Additionally, within the studies included in the analysis, there are several independent studies conducted in South Africa (n = 9), Poland (n = 7), China (n = 1), Thailand (n = 1), India (n = 1), Brazil (n = 1), Norway (n = 1), and Sweden (n = 1). In total, thirty-one genes and sixty-two genetic variations have been reported.

Risk of Bias

The NOS was utilized to assess the study's quality, and the rating greater than six indicated exceptional quality. Three papers received eight points, eleven articles received seven points, seven studies received 6 points, and only three studies were rated as of low quality (Table 2).

Influence of Genetic Factors on the ACLR

Ten studies investigated seven different collagens and ten single nucleotide polymorphisms (SNPs) with regard to the ACLR. Some studies showed the same results with no significant difference in the association of the COL1A1 rs1800012 (Gibbon et al., 2020; Manish Shukla et al., 2020; Perini et al., 2022; Sivertsen et al., 2019; Zhao et al., 2020) and rs1107946 (Gibbon et al., 2020; Perini et al., 2022) variant with the risk of ACL ruptures ($p > 0.05$). However, when individuals of European ancestry (Swedish, South African, Polish, Norwegian and Finnish; all those participants self-identified as being of white European ancestry) were combined, the rs1800012 TT genotype (TT vs. GT + GG) was significantly over-represented in the control group compared to the ACLR group ($p = 0.040$; OR = 2.8), which confirmed a strong link between rs1800012 and the ACL risk (Gibbon et al., 2020). The A allele of rs1800012 in the COL1A1 gene was more prevalent in the Norwegian than in the Finnish cohort (minor allele frequency: 0.18 vs. 0.14; $p < 0.03$) (Sivertsen et al., 2019). Perini et al. (2022) observed that the COL1A1 SNP (rs1107946, GG or TT) was a protective association with ACLR (OR = 0.25) when the three COL1A2 SNPs (rs412777, rs42524, and rs2621215) were all wildtype.

Perini et al. (2022) reported that COL1A2 rs42524 (OR = 5.73 [1.22–26.95], and rs2621215 (4.29 [1.26–14.61]) SNPs contributed significantly to the ACL risk between 146 ACLR patients and 192 healthy subjects. Sivertsen et al. (2019) showed a failed association of the COL3A1rs1800255 polymorphism and ACL injury in both Norwegian and Finnish female athletes. No COL27A1 variants were significantly associated with the risk of ACLR in South African people (European Caucasian ancestry) (Gibbon et al., 2018). Thus, there are currently insufficient evidence to support such an approach.

No significant differences were found in the genotype frequencies for the COL5A1 rs12722 polymorphism (Lulinska-Kuklik et al., 2018; Sivertsen et al., 2019; Suijkerbuijk et al., 2019; Willard et al., 2018; Zhao et al., 2020) and rs3922912, rs4841926, and rs3124299 within COL5A1 (Laguette et al., 2020). However, the

frequency distributions of allele combinations may pose a risk of the ACL injury. Willard et al. (2018) showed that when all participants or only female participants were analyzed, the COL5A1 (rs12722) and DCN (rs516115) allele combination associated with an increased risk of the ACL injury ($p = 0.006$). COL5A1-IL1B-IL6 allele T-C-G combination was significantly underrepresented ($p = 0.034$) in the Swedish male cohort control group (Suijkerbuijk et al., 2019). Contradictory evidence was revealed for COL5A1 rs13946 polymorphisms (Lulinska-Kuklik et al., 2018; Sivertsen et al., 2019; Zhao et al., 2020).

COL12A1 rs970547 and rs240736 may present a high risk of the ACLR in the Chinese male population (Zhao et al., 2020), but not in the European population (Sivertsen et al., 2019). The AA genotypes of COL12A1 rs970547 were at the level of 49.3% and 27.5% in the patient and control groups, respectively ($p = 0.026$), and rs240736 of COL12A1 played a significant role in the ACL injury in Chinese men (Zhao et al., 2020). Sivertsen et al. (2019) examined 851 female Norwegian and Finnish elite athletes and found no significant differences between the ACL injury and control groups for COL12A1 rs970547 genotypes. This suggests that COL12A1 rs970547 may increase the risk of the ACL injury in males only.

The evidence was insufficient to support the influence of MMP genes on the non-contact ACL rupture risk. Lulińska et al. (2020) found no significant differences between case and control groups for the polymorphism of MMP10 (C/T rs486055), MMP12 (T/C rs2276109), and MMP1 (-/G rs1799750). Similar results were reported for rs 679620 (A/G), rs591058 (T/C) and rs650108 (G/A) in a study by Gibbon et al. (2017). In the same cohort (Lulińska-Kuklik et al., 2019d; Rahim et al., 2019), the MMP3 rs679620 G and rs591058 C alleles were significantly over-represented in cases compared to controls (OR = 1.38 [1.05 – 1.81], $p = 0.021$), however, no association was found for MMP8 (rs11225395C/T), and TIMP2 (rs4789932 G/A) regarding the ACL injury.

Four studies (Lulinska-Kuklik et al., 2019c; Maculewicz et al., 2019; Rahim et al., 2017, 2022; Suijkerbuijk et al., 2019) indicated the lack of significant differences in the genotype and allele frequencies for IL6R rs2228145, IL6 rs1800795, IL1B rs16944 and rs1143627 when all participants (female and male) from the control and the ACL injury group were analyzed. Only the female cohort had a significantly different genotype frequency distribution for IL1B rs16944 when compared with participants in non-contact subgroups ($p = 0.039$, OR = 3.06) (Rahim et al., 2017).

Conflicting evidence was found for polymorpsims rs2010963, rs699947 of VEGFA gene in different studies. Lulińska-Kuklik et al. (2019b), Rahim et al. (2022) and Feldmann et al. (2022) found a potential correlation between the VEGFA rs2010963 (G/C) polymorphism and the ACLR risk. However, for the associations between rs699947 and the ACLR, different results were observed (Cięszczyk et al., 2017; Feldmann et al., 2022; Lulińska-Kuklik et al., 2019b; Rahim et al., 2018, 2022; Shukla et al., 2020). There were no significant differences in the genotype or allele frequency distributions for the rs1570360 of VEGFA (Feldmann et al., 2022; Lulińska-Kuklik et al., 2019b; Rahim et al., 2018, 2022). Shukla et al. (2020) observed that the ID and II genotypes, as well as the I allele (rs35569394), were associated with a 1.64-fold increased risk of the ACL rupture compared to the control group. Rahim et al. (2022) and Feldmann et al. (2022) investigated polymorphisms of the KDR gene rs2071559 A/G and rs1870377 A/T. However, their findings did not indicate that those two SNPs had an independent relationship with the ACL injury.

Two studies analyzed the relationship between the TNC gene and the ACL risk. Lulińska-Kuklik et al. (2019a) found that the genotype and allele frequencies of TNC variants (rs1330363 C/T, rs2104772 T/A, rs13321 G/C) did not differ between cases and controls. However, another study (Gibbon et al., 2018) showed that when females were examined separately, the TNC rs2104772 (A/T) variant's AA genotype was significantly associated with the ACL rupture ($p = 0.035$, OR = 2.3).

When gender was considered, conflicting results were found for the BGN rs1042103 and rs1126499 (Cięszczyk et al., 2017; Willard et al., 2018). Cięszczyk et al. (2017) observed a significant difference in the ACAN rs1516797 genotype frequencies between the control and ACLR groups ($p = 0.041$) in Polish participants. When female and male participants were analyzed together, no significant differences in the genotype and allele distributions were noted for the DCN rs516115. In

the male ACLR group, the A allele of rs1042103 (OR = 1.5) was found to be significantly over-represented, however, there were no reported significant genotype differences for the ACAN rs1516797.

Two studies investigated the effects of transforming the growth factor and tumor necrosis factor Adipokine and cytokine on ACL injury. Rahim et al. (2017) did not find any significant difference between the ACL injury and healthy groups for TGFB2 (rs7550232), TNF (rs1799964, rs1800629) and TNFRSF1B (rs1061622) when all participants (males and females) were analyzed. The TGFB rs7550232 SNP appeared to influence male weight and female height, which can provide further information to describe the genetic risk to ACL injury. Laguette et al. (2020) observed a significant difference in the frequency distribution of the rs1805113 G>A genotype (p = 0.033) between the two groups. The GG genotype was more prevalent in the control group than in the ACL group (p = 0.010, OR = 0.48). However, there were no significant differences in the genotype or allele frequency for TGFBR3 rs1805117 T > C between all groups and after sex stratification.

The limited evidence was found for the lack of association between CASP8 (rs3834129 ins/Del, rs1045485, rs13113) (Rahim et al., 2017; Seale et al., 2020), B-fibrinogenrs (rs1800789, rs1800791) (Zhao et al., 2020), PTGER4 (rs4495224) (Rahim et al., 2017) and TIMP2 (rs4789932) (Lulinska-Kuklik et al., 2019d; Rahim et al., 2019) variants and ACL injury. In addition, there was insufficient evidence to link Adiponectin rs1501299 (OR = 1.91) and B-fibrinogenrs (1800787, rs1800788, rs1800790, rs2227389) (Zhao et al., 2020) to the ACL injury.

Table 1a. Characteristics of the included studies.

Study, Year	Gene	Variant	Association?	OR (95% CI)	*p* value
Wanvisa et al., 2019	Adiponectin	rs1501299 G/T	Over-representation of the GG genotype and G allele in the injury group	1.91(1.04–3.53) 1.89(1.19–3.01)	0.026 0.004
Lulińska-Kuklik et al., 2019d	MMP3 MMP8 TIMP2	rs591058 C/T rs679620 G/A rs11225395C/T rs4789932 G/A	Over-represented rs679620 G and rs591058C alleles of MMP in the injury group	1.38(1.05–1.81)	0.021
Lulińska-Kuklik et al., 2020	MMP1 MMP10 MMP12	rs1799750 -/G rs486055 C/T rs2276109 T/C	No association		
Gibbon et al., 2017	MMP3	rs679620 A/G rs591058 T/C rs650108 G/A rs3025058 C/G	No association		
Lulińska-Kuklik et al., 2018	COL5A1	rs12722 C/T rs13946 C/T	Under-representation of the CT genotype of rs13946 in the injury group	Not shown	0.039
Shukla et al., 2020	COL1A1	rs1800012 G /T	No association		
Zhao et al., 2020	COL1A1 COL5A1 COL12A1 B-fibrinogen	rs1800012 G/T rs12722 C/T rs13946 C/T rs970547 A/G rs240736 C/T rs1800787 C/T rs1800788 C/T rs1800789 A/G rs1800790 A/G rs1800791 A/G rs2227389 C/T	Under-representation of the TT genotype of B-fib rs1800787 in the injury group; over-representation of rs1800788 CT, rs1800790 AG, and rs2227389 CT in the injury group; over-representation of the rs970547 A allele and AA genotype in the male injury group.	For rs970547 1.80 (A) 1.61 (AA	<0.05 0.019(A) 0.026(AA)

Table 1b. Characteristics of the included studies.

Study, Year	Gene	Variant	Association?	OR (95% CI)	*p* value
Laguette et al., 2020	COL5A1 TGFBR3 TGFBIT	rs3922912 G/A rs4841926 C/T rs3124299 C/T rs1805113 G/A rs1805117 T/C rs1442 G/C	 TGFBR3 rs1805113 AA vs. GG TGFBI rs1442 CC vs GG	 0.3(0.11–0.80) 0.3(0.12–0.77)	 0.017 0.013
Gibbon et al., 2020	COL1A1	rs1107946 G/T rs1800012 G/T	No association		
Perini et al., 2022	COL1A1 COL1A2	rs1107946 G/T rs412777 A/C rs42524 C/G rs2621215 G/T	COL1A2 SNPs (rs42524 CC and rs2621215 GG) were associated with an increased risk of non-contact ACL injury	5.73(1.22–26.95) 4.29(1.26–14.61)	Not shown Not shown
Sivertsen et al., 2019	COL1A1 COL3A1 COL5A1 COL12A1	rs1800012 A/C rs1107946 A/C rs1800255 A/G rs12722 C/T rs13946 C/T rs970547 C/T	No association		
Suijkerbuijk et al., 2019	COL5A1 IL1B IL6 IL6R	rs12722 C/T rs16944 C/T rs1800795 G/C rs2228145 G/C	Over-representation of the IL6R rs2228145 CC genotype in the SA-control group	Not shown	0.028
Lulińska-Kuklik et al., 2019b	VEGFA	rs699947 A/C rs1570360 A/G rs2010963 C/G	Over-representation of the VEGFA rs2010963 CC genotype in the injury group	1.85(1.11–3.08)	0.047
Lulinska-Kuklik et al., 2019c	IL1B IL6 IL6R	rs16944 G/A rs1143627 G/A rs1800795 C/G rs2228145 C/A	The rs1800795 IL6 gene polymorphism was associated with the ACL rupture	1.74(1.08–2.81)	0.010 Codominant 0.022 Recessive 0.004 Overdominant
Rahim et al., 2022	IL1B IL6 IL6R VEGFA KDR	rs16944 C/T rs1800795 G/C rs2228145 C/A rs699947 C/A rs1570360 G/A rs2010963 C/G rs2071559 A/G rs1870377 T/A	Over-representation of the VEGFA rs2010963 GC and CC genotype of rs699947 in the injury group	2.43(1.00–5.87) 3.35(1.17–9.62)	0.049 0.024

Table 1c. Characteristics of the included studies.

Study, Year	Gene	Variant	Association?	OR (95% CI)	*p* value
Rahim et al., 2022	IL1B	rs16944 C/T	Over-representation of the		
	IL6	rs1800795 G/C	VEGFA rs2010963 GC and	2.43(1.00–5.87)	0.049
	IL6R	rs2228145 C/A	CC genotype of rs699947 in	3.35(1.17–9.62)	0.024
	VEGFA	rs699947 C/A	the injury group		
		rs1570360 G/A			
		rs2010963 C/G			
	KDR	rs2071559 A/G			
		rs1870377 T/A			
Rahim et al., 2017	IL1B	rs16944 C/T	Under-representation of the		
	IL6	rs1800795 G/C	IL1B rs16944 TT genotype in	3.06(1.09–8.64)	0.039
	IL6R	rs2228145 G/C	the female control group;		
	CASP8	rs3834129 ins/del	over-representation of the		
		rs1045485 C/G	CASP8 rs3834129 ins allele in	1.46(1.01–2.12)	0.047
	TNF	rs1799964 C/T	the control group.		
		rs1800629 A/G			
	TNFRSF1B	rs1061622 G/T			
	PTGER4	rs4495224 A/C			
	TGFB2	rs7550232 A/C			
Feldmann et al., 2022	VEGFA	rs699947 C/A	Under-representation of the		
		rs1570360 G/A	VEGFA rs2010963 GG	2.8(1.45–5.41)	0.001
		rs2010963 G/C	genotype in the SWE ACL		
	KDR	rs2071559 G/A	group;		
		rs1870377 T/A	Under-representation of the	0.85(0.69–1.05)	0.010
			VEGFA AAG haplotype in		
			the combined ACL		
Seale et al., 2020	CASP8	rs3834129 ins/Del	No association		
		rs1045485 G/C			
		rs13113 T/A			
Lulińska-Kuklik et al., 2019a	TNC	rs1330363 C/T	No association		
		rs2104772 T/A			
		rs13321 G/C			
Gibbon et al., 2018	TNC	rs1061494 C/T	Under-representation of the	2.3(1.1–5.5)	0.035
		rs1138545 C/T	TNC rs2104772 AA genotype		
		rs2104772 A/T	in the female control group		
		rs1061495 C/T			
	COL27A1	rs2567706 A/G			
		rs2241671 A/G			
		rs2567705 A/T			
Willard et al., 2018	BGN	rs1126499 C/T	Allele combinations across		
		rs1042103 G/A	BGN, COL5A1 and DCN in		
	DCN	rs516115 C/T	modulating susceptibility to		
	COL5A1	rs12722 C/T	ACL injury		
Cięszczyk et al., 2017	ACAN	rs1516797 G/T	Under-representation of the		
	BGN	rs1042103 A/G	ACAN rs1516797 G/T	1.68(1.09–2.57)	0.017
		rs1126499 C/T	genotype in the control		
	DCN	rs516115 C/T	group; under-representation		
	VEGFA	rs699947 A/C	of the BGN rs1042103 A	1.5(1.05–2.15)	0.029
			allele in the male control		
			group		

Table 2. Risk of Bias Assessed by the Newcastle-Ottawa Scale.

Study	Newcastle-Ottawa Scale Score				Design
	Selection	Comparability of case	Expose	Total	
Wanvisa et al., 2019	★★★★	★★	★★	8	Case-control
Lulińska-Kuklik et al., 2019d	★★★★	★	★★	7	Case-control
Lulińska-Kuklik et al., 2020	★★★★	★	★	6	Case-control
Gibbon et al., 2017	★★★★	★	★	6	Case-control
Lulińska-Kuklik et al., 2018	★★★★	★	★★	7	Case-control
Shukla et al., 2020	★★★★	★★	★★	8	Case-control
Daohong et al., 2020	★★★★	★	★	6	Cross-sectional study
Laguette et al., 2020	★★★★	★★	★	7	Case-control
Gibbon et al., 2020	★★★	★	★	5	Case-control
Perini et al., 2022	★★★★	★	★	6	Case-control
Sivertsen et al., 2019	★★	★	★	4	Cohort study
Suijkerbuijk et al., 2019	★★★★	★★	★	7	Case-control
Lulińska-Kuklik et al., 2019b	★★★★	★	★★	7	Case-control
Rahim et al., 2018	★★★★	★★	★	7	Case-control
Shukla et al., 2020	★★★★	★	★★	7	Cross-Sectional study
Lulinska-Kuklik et al., 2019c	★★★★	★	★	6	Case-control
Rahim et al., 2022	★★★★	★	★	6	Case-control
Rahim et al., 2017	★★★★	★	★	6	Case-control
Feldmann et al., 2022	★★★	★	★	5	Case-control
Seale et al., 2020	★★★★	★	★	6	Case-control
Lulińska-Kuklik et al., 2019a	★★★★	★★	★	7	Case-control
Gibbon et al., 2018	★★★★	★★	★	7	Case-control
Willard et al., 2018	★★★★	★★	★	7	Case-control
Cięszczyk et al., 2017	★★★★	★★	★★	8	Case-control

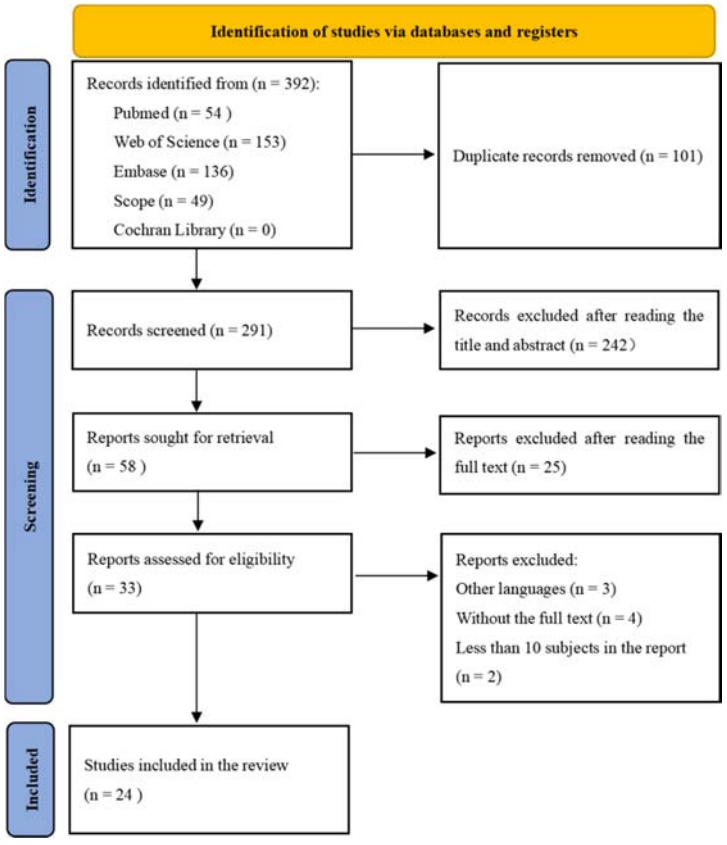

Figure 1. PRISMA flowchart showing the study-selection process.

Discussion

Researchers for a long time have been interested in whether genes have a significant impact on the ACL injury. Despite the fact that numerous studies have found a link between a genetic variation and the ACL injury, conflicting results have been reported regarding the connection between single nucleotide polymorphisms (SNPs) and the ACL rupture. According to our findings, some SNPs may contribute to the ACL risk, however, more research and a larger sample size are needed for to draw a firm conclusion. This systematic review included 24 carefully designed studies published in the last six years to summarize the potential genetic variants associated with the ACL injury, and new outcomes were presented based also on previous articles by John et al. (2016) and Kaynak et al. (2017).

The majority of research has concentrated on the genes that encode for collagens, matrix metalloproteinases, interleukins, and cell signaling molecules. Previous studies have also shown that sequence variants with these genes are associated with other musculoskeletal injuries, for instance, rotator cuff tearing (Kluger et al., 2017; Tashjian et al., 2021) and Achilles tendinopathy (Abrahams et al., 2013; Pinge et al., 2012). As a multifactorial disease, none of these genetic risk factors causes the ACL rupture on their own, but rather increases the risk of the ACL rupture in susceptible individuals. Especially when gender was taken into account, results could be different. Rahim et al. (2017) found that the IL1B rs16944 TT genotype frequency was over-represented in South African females (European Caucasian ancestry) from the ACL injury group, which is in accordance with the previous statement. According to Gibbon et al. (2018), the TNC rs2104772 polymorphism was also linked to the ACL injury in females. However, the genotype of COL12A1 rs970547 (Daohong, 2020) and KDR rs2071559 (Rahim et al., 2018) have been associated to the ACL injury only in men. The existing literature provides a contradictory theory for the influence of genetic factors on ACL injuries. The assumption that females appear to have a high genetic risk of the ACL injury (Larwa et al., 2021; Montalvo et al., 2019) can be explained by several factors, including differences in the anterior tibial translation (Myer et al., 2005), landing strategy (Yu et al., 2006), neuromuscular and kinematic control and anatomic differences between females and males (Lephart et al., 2002). However, in the genetic field this assumption needs to be corroborated by further research. While the majority of studies stratified participants based on similar age, BMI, and training levels, many studies failed to subgroup the genotype by gender, implying more research with a larger sample size, as well as gender-specific research, is needed to clarify the underlying causes and mechanisms of the ACL injury.

Clearly, genetic expression differences for the ACL injury exist among populations in various countries. In the Sivertsen et al.'s (2019) study, the C allele of the SNV in the COL12A1 gene differed between the Norwegian and Finnish cohorts, and the A allele of rs1800012 in the COL1A1 gene was more abundant in the Norwegian cohort than in the Finnish one. Shukla et al. (2020) discovered no positive association between two groups of Indian athletes for the same polymorphism. When European participants from Sweden, South Africa (European Caucasian ancestry), Poland, Norway, and Finland were combined, Gibbon et al. (2020) confirmed a strong link between rs 1800012 and the ACL risk. Various studies have found similar evidence for other polymorphisms. Researchers also attempted to demonstrate broad outcomes by analyzing the general population, as a result, bias was increased. However, when discussing the impact of genetic factors on ACL injuries, it is critical to consider nationality, especially in the initial stages of research. Hence, more research needs to be carried out around the world to support consistent proof for different ethnicities.

Another noteworthy finding is that studies by Gibbon et al. (2017, 2018, 2020) and Rahim et al. (2017, 2018, 2020) used the same group of South African participants for investigating several SNP with genes, as did Lulińska et al. (2019, 2020) for Polish participants. These findings indicate that there may be one more polymorphism associated with the ACL rupture among individuals tested in those studies, which was also emphasized in a systematic review by John et al. (2016). It is biased to focus solely on one gene when considering the impact of ACL injury.

The limitations of this systematic review were that we were unable to assess the database using meta-analysis due to the diversity of genetic

variations and the heterogeneity of the risk of bias between investigations. Additionally, some of the included studies used the same participants for analysis of different genes and polymorphisms, which was likely to increase the risk of bias in the results. When it comes to determining the risk of the ACL rupture, genetic tests can be a valuable tool, especially when it comes to assessing athletes, to determine their risk level. However, the results of candidate gene tests should only be used as part of a multifactorial risk model. To accurately assess the risk of sports injuries, we still need to identify specific genes that increase the risk of ACL injuries and use genetic screening as a diagnostic tool.

Conclusions

More research is needed to establish a clear link between the ACL rupture and genetic variants, particularly gender and nationality, that need to be considered separately. Furthermore, these findings should be validated in a larger sample of subjects from around the world.

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ORCID iD:

Zhuo Sun: https://orcid.org/0000-0003-2488-9118

Paweł Cięszczyk: https://orcid.org/0000-0002-7735-7580

Kinga Humińska-Lisowska: https://orcid.org/0000-0003-0034-0809

Monika Michałowska-Sawczyn: https://orcid.org/0000-0003-3223-1084

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Are *COL22A1* Gene Polymorphisms rs11784270 and rs6577958 Associated with Susceptibility to a Non-Contact Anterior Cruciate Ligament Injury in Polish Athletes?

Zhuo Sun [1], Paweł Cięszczyk [1], Ewelina Lulińska [1], Magdalena Dzitkowska-Zabielska [1], Monika Johne [2,*], Kinga Humińska-Lisowska [1], Monika Michałowska-Sawczyn [1], Krzysztof Ficek [3], Agata Leońska-Duniec [1], Andrzej Mastalerz [2], Arkadiusz Janczyk [4] and Sawczuk Marek [5]

[1] Faculty of Physical Education, Gdansk University of Physical Education and Sport, 80-336 Gdansk, Poland
[2] Faculty of Physical Education, Jozef Pilsudski University of Physical Education in Warsaw, 00-809 Warsaw, Poland
[3] Faculty of Physiotherapy, The Jerzy Kukuczka Academy of Physical Education in Katowice, 40-065 Katowice, Poland
[4] Center for Physiotherapy, Military Unit No. 6021, 01-001 Warsaw, Poland
[5] Institute of Physical Culture Sciences, University of Szczecin, 70-453 Szczecin, Poland
* Correspondence: monika.johne@awf.edu.pl

Abstract: Understanding the risk factors and etiology of ACL ruptures (anterior cruciate ligament) is crucial due to the injury's high occurrence, significant financial cost to the healthcare sector, and clinical consequences. In this study, we investigated the hypothesis that rs11784270 A/C and rs6577958 C/T SNPs (single gene polymorphism) within *COL22A1* are associated with ACL ruptures (ACLR) in Polish soccer players. Methods: 228 athletes with ACLR (157 male, age 26 ± 4, 71 female, age 26 ± 6) and 202 control athletes (117 male, age 26 ± 6, 85 female, age 29 ± 2) engaged in the study. The buccal cell swabs were genotyped using TaqMan® pre-designed SNP genotyping assays, following the manufacturer's recommendations. The R program and SNPassoc package were used to determine the genotype and allele frequency distributions under the various inheritance models (co-dominant, dominant, recessive, and over-dominant). Further, *p*-values of <0.05 were considered statistically significant. We found no association between the analyzed polymorphisms and the risk of non-contact ACL ruptures in any of the studied models. Although the genetic variants investigated in this study were not associated with the risk of non-contact ACL ruptures, we assumed that the *COL22A1* gene remains a candidate for further investigations in musculoskeletal injuries.

Keywords: *COL22A1* gene; injury; ACL; athletes

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1. Introduction

In sports medicine, musculoskeletal injuries (MSI) are one of the main health issues. The occurrence of the types and locations of injuries, e.g., joints, muscles, tendons, and ligaments, varies depending on the sport type and ranges from 5% to 60% [1,2].

The anterior cruciate ligament rupture (ACLR) is a frequent and serious knee injury in athletics, usually occurring without any impact [3,4]. ACL reconstruction is the preferable treatment. A total of 65% of patients typically resume their level of athletic involvement before surgery, and 55% do so at a competitive level [5,6].

Understanding the risk factors and etiology of ACL ruptures is important due to the injury's high occurrence, the significant financial cost to the healthcare sector, and the clinical consequences.

Many internal and external risk factors can result in ACL damage. Anatomical variances, sex, and genetic variability are examples of intrinsic factors [7,8]. It has been suggested that due to differences in the DNA sequence, some people may be more susceptible to ACL damage than others [9–12].

Tendons and ligaments, such as the ACL, are strands of dense regular bundles of connective tissue comprising multiple collagen fibers (such as I, III-VI, and XII). The bundles are surrounded by dense irregular connective tissue sheaths as well as various non-collagen particles, including proteoglycans and glycoproteins [13]. Collagen fibers are encoded by several genes. Collagen-type XXII alpha 1 chain, a quantitatively minor collagen of the fibril-associated collagen family with interrupted triple helices, is encoded by the *COL22A1* gene (FACITs). The biological function of this protein is not fully understood. However, it seems to support the strengthening of skeletal muscle attachments and the stabilization of myotendinous junctions (MTJ) during contractile activity [14].

In zebrafish, a loss-of-function mutation in the collagen-type XXII a-1 chain gene causes a phenotype that resembles muscular dystrophy and reduced force production. This implies that collagen is involved in the maintenance of the functional efficacy and stabilization of the MTJ [15].

In one of the studies, two SNPs in the *COL22A1* gene, namely rs11784270 and rs6577958 (the A and T alleles, respectively), were significantly associated with non-contact muscle damage in Japanese athletes [16].

Given that both the rs11784270 A/C and rs6577958 C/T SNPs located within *COL22A1* are not well described and their connection with ACL injuries is not clearly defined, we decided to test the hypothesis that they are associated with ACL susceptibility to injury in Polish athletes.

2. Materials and Methods

2.1. Participants

The study involved 430 unrelated, self-reported Caucasians recruited between the years 2009 and 2016. The anterior cruciate ligament rupture (ACLR) case group included 228 people: 157 men and 71 women. The inclusion criterion was those surgically diagnosed with primary ACLR and eligible for ligament reconstruction. The 228 subjects of the ACLR group who suffered injuries did so without physical contact. There were 202 participants in the control group (CON), 117 men and 85 women, all of whom appeared to be in good health and had never experienced an ACL injury. The ACLR males all played soccer in Poland's first, second, and third-level leagues and trained 11 to 14 h per week on average (mean time: 11.9 ± 1.4). They had an average age of 26 years and 4 months. The ACLR females (mean age: 26 ± 6 years) were soccer players from the Polish first and second-level leagues (trained 10–12 h per week; mean time: 11.1 ± 0.6). The male control group was composed of healthy, physically active adults with a mean age of 26 years and had similar amounts of exposure to sports (identical levels of training and competition intensity). The female control subjects, who self-reported being physically active for a minimum of 7 h per week (mean time 9.2 ± 1.4), were chosen from sports clubs and wellness facilities (mean age: 29 ± 2 years). Information about the study participants is summarized in Table 1.

Table 1. Characteristics of the study participants.

	N	Sex	Age	Time of Training (h/per Week)
Case group: athletes with ACL rapture	228	157 men	26 ± 4	11.9 ± 1.4
		71 women	26 ± 6	11.1 ± 0.6
	202	117 men	26 ± 6	11.2 ± 1.2

Control group: athletes without ACL rapture	85 women	29 ± 2	9.2 ± 1.4

2.2. Ethical Approval

The study's methods were approved by both the Bioethics Committee for Clinical Research of the Regional Medical Society in Gdansk (approval number KB 8/16) and the Ethics Committee of the Pomeranian Medical University in Szczecin, Poland (approval number 09/KB/IV/2011). The protocols adhered to the rules of the World Medical Association Declaration of Helsinki. An information sheet outlining the specifics of the study, including its goal, the procedures involved, as well as any risks and advantages of participation, was given to each participant. Anonymity and confidentiality were preserved in the study. All participants gave their written informed consent. The Strengthening the Publishing of Genetic Association Studies (STREGA) Statement, which establishes a set of guiding standards for reporting the findings of genetic association studies, was followed in the conduct of this case-control study [17].

2.3. Genetic Analyses

The buccal cells were collected using Copan FLOQSwabs (Copan Diagnostics, Inc., Murrieta, CA, USA) during post-surgery control. DNA was extracted using a GenElute Mammalian Genomic DNA Miniprep Kit (Sigma, Taufkirchen, Germany) in agreement with the manufacturer's instructions. On a StepOne Real-Time Polymerase Chain Reaction (RT-PCR) instrument (Applied Biosystems, Bedford, MA, USA), all samples were genotyped in duplicate using the TaqMan® pre-designed SNP genotyping assays (C_176045196_10 for the *COL22A1* rs11784270 and C_29400116_10 for the *COL22A1* rs6577958, Applied Biosystems, Bedford, MA, USA) according to the protocol [12].

2.4. Statistical Analysis

Statistical analyses were performed using the R programming environment and R package (version 3.4.0, The R Foundation for Statistical Computing, https://cran.r-project.org, accessed on 30 August 2022). Genotype and allele frequency distributions were calculated using four models of inheritance (co-dominant, dominant, recessive, and over-dominant) and the SNPassoc package 2.1-0. Further, *p*-values of <0.05 were used as a cut-off for significance.

3. Results

Tables 2 and 3 demonstrate the genotypes and allele frequency distributions for the two analyzed *COL22A1* SNPs: rs11784270 (Table 2) and rs6577958 (Table 3). In order to avoid bias in the haplotype frequency estimates, none of the polymorphisms in the co-dominant, dominant, recessive, and over-dominant models were associated with the risk of non-contact ACL ruptures. The genotype frequencies among groups are illustrated in Figure 1.

Table 2. Analysis of the relationship between non-contact ACL rupture and the *COL22A1* gene rs11784270 A/C polymorphism.

Model		CON (*n* = 202)	%	ACLR (*n* = 228)	%	OR	95% CI		*p*-Value
Co-dominant	A/A	106	52.5	114	50.0	1.00			
	A/C	69	34.2	95	41.7	1.28	0.85	1.92	0.119
	C/C	27	13.4	19	8.3	0.65	0.34	1.25	
Dominant	A/A	106	52.5	114	50.0	1.00			0.608
	A/C-C/C	96	47.5	114	50.0	1.10	0.76	1.61	

Recessive	A/A-	175	86.6	209	91.7	1.00			0.092
	C/C	27	13.4	19	8.3	0.59	0.32	1.10	
Over-dominant	A/A-	133	65.8	133	58.3	1.00			0.109
	A/C	69	34.2	95	41.7	1.38	0.93	2.04	
Allele	A	281	69.6	323	70.8	1.00			0.682
	C	123	31.4	133	29.2	1.06	0.79	1.42	

ACLR—anterior cruciate ligament rupture, CON—control, OR—odds ratio, 95% CI—confidence intervals.

Table 3. Association analysis of the *COL22A1* gene rs6577958 T/C polymorphism with non-contact ACL rupture.

Model		CON (*n* = 202)	%	ACLR (*n* = 228)	%	OR	95% CI		*p*-Value
Co-dominant	T/T	131	65.2	150	65.8	1.00			
	C/T	53	26.4	67	29.4	1.10	0.72	1.70	0.284
	C/C	17	8.5	11	4.8	0.57	0.26	1.25	
Dominant	T/T	131	65.2	150	65.8	1.00			0.894
	C/T-C/C	70	34.8	78	34.2	0.97	0.65	1.45	
Recessive	T/T-C/T	184	91.5	217	95.2				0.128
	C/C	17	8.5	11	4.8	0.55	0.25	1.20	
Over-dominant	T/T-C/C	148	73.6	161	70.6				0.487
	C/T	53	26.4	67	29.4	1.16	0.76	0.76	
Allele	T	315	78.4	367	80.5	1.00			
	C	87	21.6	89	19.5	1.14	0.82	1.59	0.442

ACLR—anterior cruciate ligament rupture, CON—control, OR—odds ratio, 95% CI—confidence intervals.

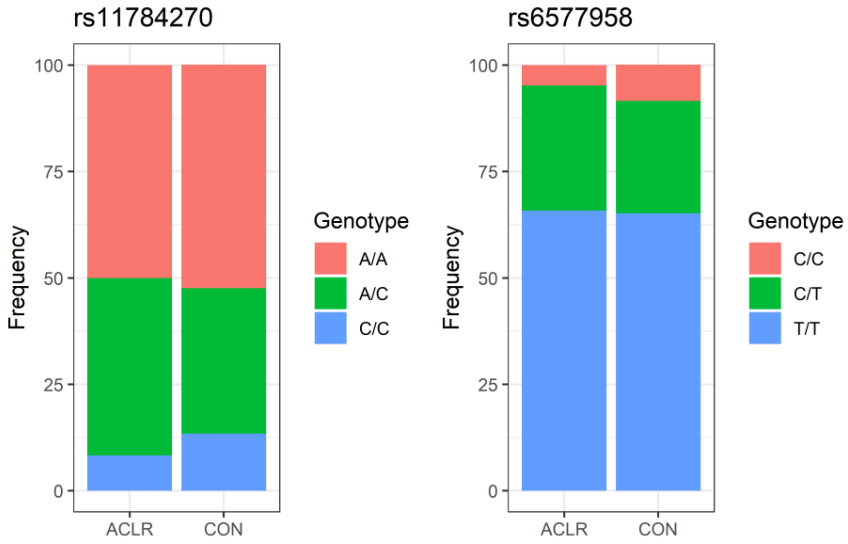

Figure 1. Genotypes frequency in ACLR and control groups.

4. Discussion

This study aimed to investigate the association polymorphism variants located within *COL22A1* with ACL injuries. The main findings of this genetic association study show (i) no independent associations between the rs11784270 A/C and rs6577958 C/T polymorphisms and non-contact ACL ruptures; (ii) none of the polymorphisms in the co-

dominant, dominant, recessive, and over-dominant models were associated with the risk of non-contact ACL ruptures.

The *COL22A1* gene encodes COLXXII, FACIT-type collagen, and its biological role has not been fully understood. This protein has been described as a unique tissue junction element found predominantly in transitional tissue zones, e.g., the MTJ of the skeletal muscle, the heart, the articular cartilage-synovial fluid junction, and the junction between the hair follicle and dermis [18,19]. Zebrafish COLXXII depletion resulted in muscular dystrophy, probably due to a disrupted myotendinous junction [15]. In addition, adult homozygous mutants had a higher frequency of cerebral hemorrhages due to increased vascular permeability [20]. It has recently been shown that *COL22A1* is involved in mice bone remodeling and is expressed in bone-forming osteoblasts, but not bone-resorbing osteoclasts—*COL22A1*-deficient mice displayed trabecular osteopenia as well as a significantly increased osteoclast number [19]. In humans, *COL22A1* genetic variants have been linked to a higher risk of aneurysms and are being investigated for their potential contribution to musculoskeletal soft tissue injuries. Specifically, it has been shown that SNPs associated with different mRNA expression levels of the collagen-type XXII gene (rs11784270 and rs6577958) are related to athlete susceptibility to muscle injuries. The high expression of A (rs11784270) and T (rs6577958) alleles were strongly linked to muscle damage in athletes when analyzed using additive genetic models. According to the authors, a high expression of *COL22A1* at the MTJ is linked to muscle injury risk in athletes [16].

Our genetic association study set out to determine the possible interaction between these two SNPs within the *COL22A1* gene (rs11784270 and rs6577958) and non-contact ACL ruptures in a Polish cohort. However, under the co-dominant, dominant, recessive, and over-dominant models, we found no independent associations between the rs11784270 and rs6577958 polymorphisms and non-contact ACL tears.

Since the available literature concerning the role of COLXXII in ACL formation and remodeling is unclear, it is difficult to determine whether the *COL22A1* polymorphisms are associated with injury risk to the ACL.

We chose a genetic marker for the study based on the assumption that tendons and ligaments have similar basic structures and gene expression patterns of their major cell types [13].

Ligaments are bands of connective tissue made of collagenous fibers that connect bones to other bones to form joints, while tendons connect bones to muscles. The key factors affecting the mechanical characteristics of ligaments and their subsequent structural integrity have been demonstrated to be the collagen content and cross-linking between collagen fibers [21–23]. In healthy ligaments, collagen replacement occurs. This manifests in collagen fibrils' cross-linking maturation and elevated tensile strength [23,24]. The high collagen turnover in such ligaments is maintained in part by mechanical incentives [25,26]. For instance, exercise has been demonstrated to cause an increase in the synthesis of ligament collagen [24,27].

To date, several studies, including our own, have shown that a variation in collagen coding genes contributes to ACL injury risk. These genes were *COL1A1*, *COL3A1*, *COL5A1*, and *COL12A1,* which were associated with ACL rupture [28–33].

The regulation of expression and the appropriate proportion of collagen fibers that build ligaments and tendons are crucial for their integrity. For example, a higher expression of the main collagenous component of the ligaments and tendons, *COL1A1*, might raise the possibility of ACL injury by disrupting the structural integrity of collagen fibers [28,34]. The most common *COL1A1* variant, rs1800012 (+1245G/T, Sp1), results in the G allele's substitution with the T allele within the gene's first intron. The rs1800012 SNP leads to an increase in the levels of type I collagen in α1 chains. In turn, this results in a disproportion in the ratio between the α1 and α2 chains (2:1) by increasing the binding affinity of RNA polymerase II [12,35–37].

A higher *COL22A1* expression caused by the A and T alleles of rs11784270 and rs6577958, respectively, has been associated with muscle injury in athletes. It has been postulated that high levels of *COL22A1* expression at the MTJ alter the MTJ properties [16]. The rs11784270 and rs679620 variants are detected within the intron of *COL22A1*. According to the GTEx Portal (https://www.gtexportal.org/home, accessed on 30 August 2022), the A and T alleles have higher transcriptional activity than the C and C alleles of the rs11784270 and rs679620, respectively [16]. However, there is currently an insufficient understanding of the molecular mechanisms that determine the high expression of the *COL22A1* A and C alleles. Therefore, efforts should be made to better understand the process underlying *COL22A1* expression alteration. Despite the fact that the SNPs analyzed in the present study were not associated with the risk of non-contact ACL rupture, we assumed that the *COL22A1* gene remains an important biological candidate for further interrogation of musculoskeletal injuries based on the findings of earlier case-control genetic association studies [16,28].

5. Conclusions

Despite the fact that the genetic variations examined in this study were not linked to the risk of non-contact ACL injury, we inferred from earlier case-control genetic association studies that the *COL22A1* gene is still a crucial biological candidate for further investigation in musculoskeletal injuries.

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

Zhuo Sun
Gdansk university of physical education and sport, zhuo.sun@awf.gda.pl

Aleksandra Bojarczuk
Gdansk University of Physical Education and Sport, aleksandra.bojarczuk@awf.gda.pl

Pawel Cieszczyk
Gdańsk University of Physical Education and Sport, pawel.cieszczyk@awf.gda.pl

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Abstract

Introduction: Sports injuries are the most common cause of anterior cruciate ligament (ACL) ruptures. Previous research has demonstrated links between some of the *COL27A1* and *COL11A1* genetic variants and musculoskeletal soft tissue injuries. However, no previous research has investigated the *COL27A1* rs1570460 and the *COL11A1* rs3753841 in the context of ACL rupture in any population. Thus, our study aimed to assess the association between specific single nucleotide polymorphisms (SNPs), i.e., COL27A1 rs946053 and COL11A1 rs3753841, and the occurrence of ACL injury (ACL-I) in a cohort of Polish athletes. Methods: The study enrolled 233 athletes with ACL-I (161 males and 71 females) and 228 healthy control athletes (143 males and 85 females) with no prior ACL-I history. Genotyping was conducted to assess the presence of *COL27A1* rs946053 and *COL11A1* rs3753841 genetic variants. Statistical analyses were performed using the R programming environment and package, and an association between SNPs and ACL-I was tested in four genetic models: dominant, co-dominant recessive, and over-dominant. Results: All the analyzed polymorphisms conformed to Hardy-Weinberg equilibrium (HWE). The study revealed no significant differences between the ACL-I and control groups. Conclusions: Despite the absence of significant associations between the investigated SNPs and ACL-I in this study, our findings highlight the importance of continued research to unravel the precise genetic risk mechanisms and etiological factors contributing to ACL-I.

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

Zhuo SUN[1]*, Aleksandra BOJARCZUK[2], Paweł CIĘSZCZYK[3]

[1] Gdansk University of Physical Education and Sport, Gdansk, Poland; ORCID 0000-0003-2488-9118
[2] Gdansk University of Physical Education and Sport, Gdansk, Poland; ORCID 0000-0002-4398-3815
[3] Gdansk University of Physical Education and Sport, Gdansk, Poland; ORCID 0000-0002-7735-7580

* Correspondence: Zhuo Sun, Gdansk University of Physical Education and Sport, 1 Gorskiego St, 80-336 Gdansk, Poland; e-mail: huo.sun@awf.gda.pl

Abstract: Introduction: Sports injuries are the most common cause of anterior cruciate ligament (ACL) ruptures. Previous research has demonstrated links between some of the *COL27A1* and *COL11A1* genetic variants and musculoskeletal soft tissue injuries. However, no previous research has investigated the *COL27A1* rs1570460 and the *COL11A1* rs3753841 in the context of ACL rupture in any population. Thus, our study aimed to assess the association between specific single nucleotide polymorphisms (SNPs), i.e., *COL27A1* rs946053 and *COL11A1* rs3753841, and the occurrence of ACL injury (ACL-I) in a cohort of Polish athletes. Methods: The study enrolled 233 athletes with ACL-I (161 males and 71 females) and 228 healthy control athletes (143 males and 85 females) with no prior ACL-I history. Genotyping was conducted to assess the presence of *COL27A1* rs946053 and *COL11A1* rs3753841 genetic variants. Statistical analyses were performed using the R programming environment and package, and an association between SNPs and ACL-I was tested in four genetic models: dominant, co-dominant recessive, and over-dominant. Results: All the analyzed polymorphisms conformed to Hardy-Weinberg equilibrium (HWE). The study revealed no significant differences between the ACL-I and control groups. Conclusions: Despite the absence of significant associations between the investigated SNPs and ACL-I in this study, our findings highlight the importance of continued research to unravel the precise genetic risk mechanisms and etiological factors contributing to ACL-I.

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

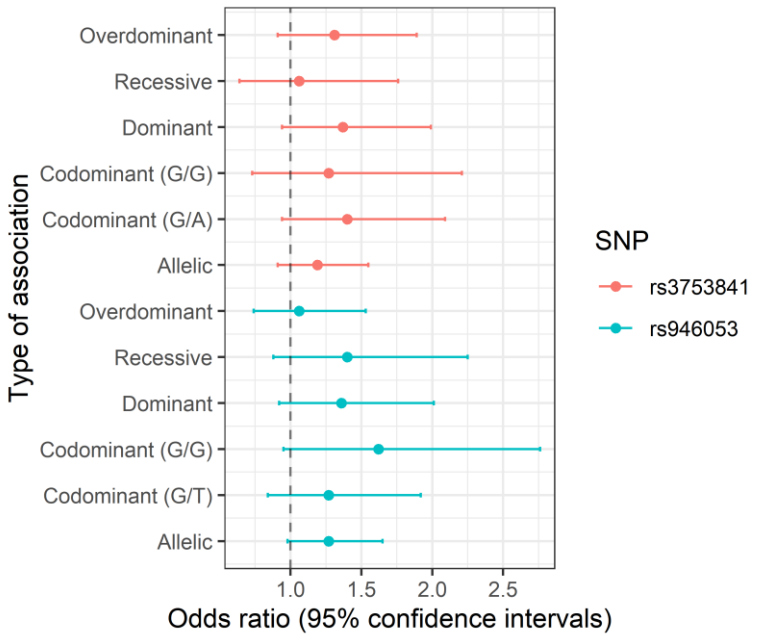

Figure 1. Odds ratios and 95% confidence intervals for the allelic and genotype-based associations

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.

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Data Availability Statement: Data available from the corresponding author on request.

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