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

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Keywords

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

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

Zhuo SUN*, Aleksandra BOJARCZUK, Paweł CIĘSZCZYK

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.

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 α1 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* polymorphism rs94605 and the *COL11A1* rs3753841 polymorphism 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. Participants

This study involved 461 unrelated self-reported Caucasian participants between 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

<table>
<thead>
<tr>
<th>Group</th>
<th>N (461)</th>
<th>Gender</th>
<th>Age (SD ± X)</th>
<th>Time of Training (h/week)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACL</td>
<td>233</td>
<td>Male 161</td>
<td>26 ± 4</td>
<td>11.7 ± 1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female 71</td>
<td>26 ± 6</td>
<td>11.1 ± 0.6</td>
</tr>
<tr>
<td>CON</td>
<td>228</td>
<td>Male 143</td>
<td>26 ± 6</td>
<td>11.2 ± 1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female 85</td>
<td>26 ± 2</td>
<td>9.2 ± 1.4</td>
</tr>
</tbody>
</table>

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., codominant, 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

<table>
<thead>
<tr>
<th>SNP</th>
<th>MAF</th>
<th>HWE (p-value)</th>
<th>Missing (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs946053</td>
<td>T (56.7%)</td>
<td>0.924</td>
<td>0</td>
</tr>
<tr>
<td>rs3753841</td>
<td>A (61.7%)</td>
<td>0.492</td>
<td>0</td>
</tr>
</tbody>
</table>

MAF – minor allele frequency

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

<table>
<thead>
<tr>
<th>SNP</th>
<th>Control + Cases</th>
<th>Controls</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs946053</td>
<td>0.924</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>rs3753841</td>
<td>0.492</td>
<td>0.256</td>
<td>0.895</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Allele (rs946053)</th>
<th>Control</th>
<th>Cases</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>278 (53.2%)</td>
<td>245 (46.8%)</td>
<td>523</td>
</tr>
<tr>
<td>G</td>
<td>188 (47.1%)</td>
<td>211 (52.9%)</td>
<td>399</td>
</tr>
<tr>
<td>Total</td>
<td>466</td>
<td>456</td>
<td>922</td>
</tr>
</tbody>
</table>

Table 5. Allelic contingency table for the rs3753841 polymorphism

<table>
<thead>
<tr>
<th>Allele (rs3753841)</th>
<th>Control</th>
<th>Cases</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>297 (63.7%)</td>
<td>272 (59.6%)</td>
<td>569</td>
</tr>
<tr>
<td>G</td>
<td>169 (36.3%)</td>
<td>184 (40.4%)</td>
<td>353</td>
</tr>
<tr>
<td>Total</td>
<td>466</td>
<td>456</td>
<td>922</td>
</tr>
</tbody>
</table>

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-1 under the four models.
Table 6. Analysis of the genetic relationship between the COL27A1 gene rs946053 G/T polymorphism and non-contact ACL rupture

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

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

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

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 LOC105376225 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. Besides, 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 between 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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