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## Genetic variants and anterior cruciate ligament rupture – Elastin proteins gene and fibromodulin gene polymorphisms

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### Abstract

Introduction: Anterior cruciate ligament (ACL) injuries are among the most common ligament injuries of the knee and often require reconstruction. The etiology of the molecular mechanisms of rupture of the anterior cruciate ligament (ACL) is poorly understood, and many external and internal factors have been associated with it, including genetics. This study aimed to investigate the association of sequence variants in the *ELN* and *FMOD* candidate genes with ACL ruptures based on the essential biological functions of these two proteoglycan-encoding genes in maintaining the structural integrity of tissues and regulating fibrillogenesis. Material and Methods: The participants consisted of 229 (164 male and 65 female) individuals with a surgically diagnosed ACL rupture (ACLR) who qualified for ligament reconstruction and 192 (107 male and 85 female) healthy participants with no history of ACL injury (CON group). All 229 participants in the ACLR group sustained injuries through non-contact mechanisms. The chi-square tests were used for association analysis of *ELN rs2071307* and *FMOD rs7543148* between the anterior cruciate ligament rupture (ACLR) group and the control (CON) group. Results: It follows from the study that the tested group of athletes shows a statistically significantly more frequent AA genotype in the *ELN rs2071307* gene polymorphism than the control group. However, looking at gender, one can observe that these are significantly more frequent. In the polymorphism of the *FMOD rs7543148* gene, a significantly rarer TT genotype was observed in the study group compared to the control group and, similarly to the previous polymorphism, the TT genotype was significantly less frequent in the group of males compared to the control group. Conclusions: Genetic research in sports, especially concerning injuries connected to characteristic polymorphic variants of selected genes, is justified, particularly as regards gender-homogenous groups.

### Keywords

elastin proteins, fibromodulin gene, anterior cruciate ligament rupture

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Article

# Genetic variants and anterior cruciate ligament rupture – Elastin proteins gene and fibromodulin gene polymorphisms

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**Abstract:** Introduction: Anterior cruciate ligament (ACL) injuries are among the most common ligament injuries of the knee and often require reconstruction. The etiology of the molecular mechanisms of rupture of the anterior cruciate ligament (ACL) is poorly understood, and many external and internal factors have been associated with it, including genetics. This study aimed to investigate the association of sequence variants in the *ELN* and *FMOD* candidate genes with ACL ruptures based on the essential biological functions of these two proteoglycan-encoding genes in maintaining the structural integrity of tissues and regulating fibrillogenesis. Material and Methods: The participants consisted of 229 (164 male and 65 female) individuals with a surgically diagnosed ACL rupture (ACLR) who qualified for ligament reconstruction and 192 (107 male and 85 female) healthy participants with no history of ACL injury (CON group). All 229 participants in the ACLR group sustained injuries through non-contact mechanisms. The chi-square tests were used for association analysis of *ELN rs2071307* and *FMOD rs7543148* between the anterior cruciate ligament rupture (ACLR) group and the control (CON) group. Results: It follows from the study that the tested group of athletes shows a statistically significantly more frequent AA genotype in the *ELN rs2071307* gene polymorphism than the control group. However, looking at gender, one can observe that these are significantly more frequent. In the polymorphism of the *FMOD rs7543148* gene, a significantly rarer TT genotype was observed in the study group compared to the control group and, similarly to the previous polymorphism, the TT genotype was significantly less frequent in the group of males compared to the control group. Conclusions: Genetic research in sports, especially concerning injuries connected to characteristic polymorphic variants of selected genes, is justified, particularly as regards gender-homogenous groups.

**Keywords:** elastin proteins, fibromodulin gene, anterior cruciate ligament rupture.

## 1. Introduction

Anterior cruciate ligament (ACL) injuries are among the most common ligament injuries of the knee and often require reconstruction [1,2]. The etiopathogenesis of these injuries mainly focuses on the mechanism of injury, the patient's sex, and anatomical factors as predisposing factors. Most ACL tears are caused by the mechanism of non-contact valgus hyperextension, although direct trauma may also be the cause. Various intrinsic and extrinsic risk factors predispose a person to ACL rupture, although knee injury is necessary. External risk factors include the playing surface, type of sport, activity level, weather conditions, type of footwear, and protective equipment used. Intrinsic risk factors include age, gender, anatomical risk factors (e.g., knee geometry, notch width, Q angle, tibial slope, pelvic tilt, generalized/anterior knee laxity, body mass index, foot pronation, ACL size), neuromuscular and cognitive factors, hormonal factors and genetic factors [3,4].

The etiology of the molecular mechanisms of rupture of the anterior cruciate ligament (ACL) is poorly understood, and many external and internal factors have been associated with it, including genetics [5–7]. Polymorphic variants within several collagen-encoding genes that regulate the formation of collagen fibrils, the basic building blocks of ligaments, are associated with ACL rupture [7–10]. In sports that require rapid changes of direction and/or release, such as cuts, spins, and landings, the risk of ACL rupture is increased [11]. In addition, proteoglycans, biglycan, decorin, fibromodulin, and lumican play an essential structural role in ligaments and regulate fibrillogenesis [12]. These components work together to improve the knee's dynamic, flexible and biomechanical properties for practical function.

At first, we examined the polymorphism of the elastin protein ELN gene, taking into account its roles and clinical significance. Elastin proteins ELN contribute to the elasticity of tendons and ligaments, allowing them to stretch and return to their original state. ELNs play an important supporting role in musculoskeletal tissues and are expressed in places where mechanical energy is stored [13]. The rs2071307 ELN gene variant is associated with other multifactorial ECM conditions, such as aortic stenosis [10] and aortic aneurysm [14]. Interestingly, the rs2071307 ELN variant is located in exon 20 of the gene and is a non-synonymous SNP. It is predicted to be harmful because it replaces the hydrophobic amino acid glycine with a hydrophilic serine residue. This substitution can disrupt the integrity of microfibrils, making them more susceptible to damage [18]. This variant may predispose people to soft tissue damage during physical activity [15].

As we know, ACL injury is polygenic and multifactorial; therefore, the genetic analysis of the fibromodulin gene (FMOD) was chosen in this study. The FMOD fibromodulin gene is located on chromosome 1q32 [16]. Fibromodulin belongs to the family of leucine-rich proteoglycans [17]. It was first identified as a collagen-binding molecule widely distributed in connective tissues, especially cartilage [18]. However, *FMOD* gene has not been associated with any multifactorial conditions until today [16].

This study aimed to investigate the association of sequence variants in the ELN and FMOD candidate genes with ACL ruptures based on the essential biological functions of these two proteoglycan-encoding genes in maintaining the structural integrity of tissues and regulating fibrillogenesis.

## 2. Materials and Methods

### 2.1. Participants

A total of 421 physically active, unrelated Caucasian, self-referred participants were recruited for this follow-up genetic association study between 2009 and 2016. These participants consisted of 229 (164 male and 65 female) individuals with a surgically diagnosed ACL rupture (ACLR) who qualified for ligament reconstruction and 192 (107 male and 85 female) healthy participants with no history of ACL injury (CON group). All 229 partici-

pants in the ACLR group sustained injuries through non-contact mechanisms. The participants of the ACLR were footballers (164 men and 65 women) from the Polish 1st, 2nd and 3rd football leagues (training for 11–14 hours a week). The control group consisted of healthy participants, mainly football players (107 men and 85 women), with no history of ligament or tendon injuries. All male participants (ACLR and CON groups) were from the same football teams, of the same ethnicity (self-reported, all Poles, Eastern Europeans  $\geq 3$  generations), of similar age (ACLR group =  $26 \pm 4$  years old, the control group =  $25 \pm 3$  years old) and had a comparable level of exposure to the risk of ACL injury (same volume and intensity of training and match play). The ACLR participants (aged  $25 \pm 4$  years) were football players of the 1st league of Polish football (training for 10–12 hours a week) and amateur skiers. Participants from the CON group (aged  $29 \pm 2$  years) were recruited from sports clubs and wellness centres (physical activity of minimum 7 hours a week).

## 2.2. Procedure

The procedures used in the study were ethically conducted following the World Medical Association's Declaration of Helsinki and ethical standards in sport and exercise research. The Pomeranian Medical University Ethics Committee approved the study in Szczecin (approval number 09/KB/IV/2011). All participants received written information about the study, including the purpose, procedures, risks, and benefits from participation. After assuring the participant had understood the provided information, each gave written informed consent. Experimental procedures were performed following the Strengthening the Reporting of Genetic Association studies (STREGA) statement.

DNA was extracted from buccal cells with sterile foam tip applicators (Puritan, USA) using the GenElute Mammalian Genomic DNA Miniprep Kit (Sigma, Germany) according to the manufacturer's protocol. To discriminate *ELN* rs2071307 and *FMOD* rs7543148 alleles, a TaqMan Pre-Designed SNP Genotyping Assay (Applied Biosystems) (assay ID: C 8787735 1, C 16182844 10, C 11844394 10, respectively) was used, and all samples were genotyped in duplicate on a StepOne Real-Time Polymerase Chain Reaction (RT-PCR) instrument (Applied Biosystems, USA).

## 2.3. Statistical Analysis

The chi-square tests were used for association analysis of *ELN* rs2071307 and *FMOD* rs7543148 between the anterior cruciate ligament rupture (ACLR) and control (CON) groups. All calculations were performed using STATISTICA 13 (Tibco Software Inc., Palo Alto, CA, USA) for Windows (Microsoft Corporation, Redmond, WA, USA) and the CATT package in R for Cochran-Armitage trend test.

## 3. Results

The frequency distributions accorded with the HWE (Hardy-Weinberg Equilibrium). A statistically significant deviation from the Hardy-Weinberg Equilibrium law was demonstrated in men with ACLR for the *ELN* rs2071307 polymorphism. However, for all people with ACLR and for women with ACLR and the control group, as well as for the control group by sex, the frequency of the *ELN* rs2071307 polymorphism does not differ from the Hardy-Weinberg Equilibrium law. Also, for the *FMOD* rs7543148 polymorphism, a deviation from the Hardy-Weinberg Equilibrium law was observed in all ACLR subjects and in ACLR males. However, in women with ACLR, the control group, and the control group, the frequency of the *FMOD* rs7543148 polymorphism does not differ from the Hardy-Weinberg Equilibrium law (Table 1).

The analysis of the associations of *ELN* rs2071307 polymorphisms of the examined group of all participants between the anterior cruciate ligament rupture (ACLR) group and the control (CON) group showed statistically insignificant differences (G / G 22.4% vs. G / G 37.2%; G / A 56.1% vs. G / A 50.8%, A / A 21.5% vs. A / A 12%,  $\chi^2 = 13.78$ ;  $p = 0.00109$ ). Statistically significant differences in incidence were observed in alleles for *ELN*

*rs2071307* between all the anterior cruciate ligament rupture (ACLR) groups and the control (CON) group (G 50.4% vs. G 62.6%, A 49.6% vs. A 37.4%,  $\chi^2 = 12.40$ ,  $p = 0.00043$ , Table 1). The analysis of the associations of *ELN rs2071307* polymorphisms of the examined group of male participants between the anterior cruciate ligament rupture (ACLR) group and the control (CON) group showed statistically insignificant differences (G / G 18.4% vs. G / G 37.4%; G / A 57.7% vs. G / A 53.3%, A / A 23.9% vs. A / A 9.3%,  $\chi^2 = 16.76$ ;  $p = 0.00023$ ). Statistically significant differences in incidence were observed in alleles for *ELN rs2071307* between the male anterior cruciate ligament rupture (ACLR) group and the control (CON) group (G 47.2% vs. G 58.9%, A 52.8% vs. A 41.1%,  $\chi^2 = 7.01$ ,  $p = 0.00810$ , Table 1). The analysis of the associations of *ELN rs2071307* polymorphisms of the examined group of female participants between the anterior cruciate ligament rupture (ACLR) group and the control (CON) group showed statistically insignificant differences (G / G 32.3% vs. G / G 36.9%; G / A 52.3% vs. G / A 47.6%, A / A 15.4% vs. A / A 15.5%,  $\chi^2 = 0.38$ ;  $p = 0.82518$ ). Statistically significant differences in incidence were observed in alleles for *ELN rs2071307* between the female anterior cruciate ligament rupture (ACLR) group and the control (CON) group (G 58.5% vs. G 60.7%, A 41.5% vs. A 39.3%,  $\chi^2 = 0.15$ ,  $p = 0.69415$ , Table 1).

The analysis of the associations of *FMOD rs7543148* polymorphisms of the examined group of all participants between the anterior cruciate ligament rupture (ACLR) group and the control (CON) group showed statistically insignificant differences (C / C 9.2% vs. C / C 4.2%; C / T 29.8% vs. C / T 24.6%, T / T 61% vs. T / T 71.2%,  $\chi^2 = 6.48$ ;  $p = 0.03919$ ). Statistically significant differences in incidence were observed in alleles for *FMOD rs7543148* between all the anterior cruciate ligament rupture (ACLR) groups and the control (CON) group (C 24.1% vs. C 16.5%, T 75.9% vs. T 83.5%,  $\chi^2 = 5.90$ ,  $p = 0.01502$ , Table 1). The analysis of the associations of *FMOD rs7543148* polymorphisms of the examined group of male participants between the anterior cruciate ligament rupture (ACLR) group and the control (CON) group showed statistically insignificant differences (C / C 10.4% vs. C / C 2.8%; C / T 28.8% vs. C / T 21.5%, T / T 60.7% vs. T / T 75.7%,  $\chi^2 = 8.58$ ;  $p = 0.01358$ ). Statistically significant differences in incidence were observed in alleles for *FMOD rs7543148* between the male anterior cruciate ligament rupture (ACLR) group and the control (CON) group (C 24.8% vs. C 15%, T 75.2% vs. T 85%,  $\chi^2 = 7.64$ ,  $p = 0.00570$ , Table 1). The analysis of the associations of *FMOD rs7543148* polymorphisms of the examined group of female participants between the anterior cruciate ligament rupture (ACLR) group and the control (CON) group showed statistically insignificant differences (C / C 6.2% vs. C / C 6%; C / T 32.3% vs. C / T 28.6%, T / T 61.5% vs. T / T 65.4%,  $\chi^2 = 0.26$ ;  $p = 0.87768$ ). Statistically significant differences in incidence were observed in alleles for *ELN rs2071307* between the female anterior cruciate ligament rupture (ACLR) group and control (CON) group (C 22.3% vs. C 20.2%, T 77.7% vs. T 79.8%,  $\chi^2 = 0.19$ ,  $p = 0.66424$ , Table 1).

**Table 1.** Genotype and minor allele frequency distributions of the *ELN rs2071307* and *FMOD rs7543148* variants in all participants as well as male and female participants only between the control (CON) group and anterior cruciate ligament rupture (ACLR) group in a Polish Caucasian cohort.

		All			Male			Female		
		ACLR	CON	$\chi$ (p-value)	ACLR	CON	$\chi$ (p-value)	ACLR	CON	$\chi$ (p-value)
<i>ELN</i> rs2071307	n	228	191	13.78	163	107	16.76	65	84	0.38
	GG	51 (22.4%)	71 (37.2%)	0.00109	30 (18.4%)	40 (37.4%)	0.00023	21 (32.3%)	31 (36.9%)	0.82518
	GA	128 (56.1%)	97 (50.8%)		94 (57.7%)	57 (53.3%)		34 (52.3%)	40 (47.6%)	
	AA	49 (21.5%)	23 (12%)		39 (23.9%)	10 (9.3%)		10 (15.4%)	13 (15.5%)	
	G allele	230 (50.4%)	239 (62.6%)	12.40	154 (47.2%)	126 (58.9%)	7.01	76 (58.5%)	102 (60.7%)	0.15
	A allele	226 (49.6%)	143 (37.4%)	0.00043	172 (52.8%)	88 (41.1%)	0.00810	54 (41.5%)	66 (39.3%)	0.69415
	HWE	3.44	1.35		4.01	2.61		0.39	0.0003	
$\chi$ (p-value)	0.063503	0.244674		0.045158	0.105885		0.534746	0.986965		
<i>FMOD</i> rs7543148	N	228	191	6.48	163	107	8.58	65	84	0.26
	CC	21 (9.2%)	8 (4.2%)	0.03919	17 (10.4%)	3 (2.8%)	0.01358	4 (6.2%)	5 (6%)	0.87768
	CT	68 (29.8%)	47 (24.6%)		47 (28.8%)	23 (21.5%)		21 (32.3%)	24 (28.6%)	
	TT	139 (61%)	136 (71.2%)		99 (60.7%)	81 (75.7%)		40 (61.5%)	55 (65.4%)	
	C allele	110 (24.1%)	63 (16.5%)	5.9	81 (24.8%)	32 (15%)	7.64	29 (22.3%)	34 (20.2%)	0.19
	T allele	346 (75.9%)	319 (83.5%)	0.01502	245 (75.2%)	182 (85%)	0.00570	101 (77.7%)	134 (79.8%)	0.66424
	HWE	7.83	2.17		8.47	0.72		0.30	1.11	
$\chi$ (p-value)	0.005146	0.140564		0.003615	0.393031		0.583857	0.291831		

#### 4. Discussion

It can be seen from the obtained study results that the tested group of athletes in the *ELN rs2071307* gene polymorphism shows a statistically significantly more frequent AA genotype than the control group (Table 1). However, looking at gender, these significantly more frequent differences occur only in the group of men compared to the male control group (Table 1).

A significantly rarer genotype was observed in the study group in the same polymorphism compared to the control group. As before, it was significantly less frequent in the male study group compared to the male control group.

However, in the polymorphism of the *FMOD rs7543148* gene, a significantly rarer TT genotype was observed in the study group compared to the control group and, similarly to the previous polymorphism, the TT genotype was significantly less frequent in the group of males compared to the male control group.

As can be seen in the presented results, although only two genes and their polymorphic variants were studied, statistically significant results were obtained, especially in the group of men.

It is widely acknowledged that some individuals are naturally gifted with specific physical attributes that, in turn, are related to athletic performance [19]. In some cases, gifted individuals come from talented families, suggesting that genetics is partly responsible for the physical, physiological or anthropometric characteristics needed for athletic success [20]. Indeed, an elite athlete status is partly inherited, with twin studies suggesting that 30–80% of the variance in this trait is explained by hereditary factors [21].

Due to the nature of the competition and the physiological demands placed on the body, there is an inherent risk of injury for athletes [22–24]. Just as much as we are interested in the genetic determinants of sports championships, we are, or maybe even more, interested in whether there are genetic determinants of the risk of injury in an athlete.

Ligament injuries are common, especially in the ACL (25–50% of knee ligament injuries; incidence rate 1 in 3,000 population per year in the United States and Europe) [25]. ACL injuries mainly result from sports activity (65%) in the young population (70% of affected patients aged 20–35) and occur as chronic tissue degeneration in the ageing population, resulting in a weakening of the musculoskeletal system and potentially leading to osteoarthritis [25,26].

Over the past 2 decades, several researchers have studied genetic factors that may predispose to ACL rupture. These tests have evolved from simple familial predisposition tests to more complex gene association studies [27–43]. Most studies of genetic predisposition to ACL rupture have focused on single nucleotide polymorphisms (SNPs). SNPs are the most common type of genetic variability observed among individuals; each SNP represents a single nucleotide difference. Type 1 collagen is the main component of ACL; therefore, early studies looked at SNPs involving the *COL1* (type 1 collagen) gene [39, 45]. Other genes have also been investigated in later studies, including the *COL12*, *COL5*, *COL3*, *COL6*, *MMP* (matrix metalloproteinase) and *ECM* (extracellular matrix) genes [46].

Of course, it should be emphasized that ACL is not conditioned by monogenic and unifactorial factors. Many genes that predispose to the risk of ACL injuries have been studied. It is interesting to see how different genes and their polymorphisms have been studied in this regard. Posthumus et al. [37] conducted a study to determine whether variant *Bst*UI and *Dpn*II [RFLP] restriction fragment length polymorphisms within the *COL5A1* gene are associated with an increased risk of anterior cruciate ligament rupture and (2) whether there are any positive sex-specific associations between the 2 sequence variants *COL5A1* and the risk of anterior cruciate ligament rupture. In total, they examined 129 people diagnosed with ACL injuries and 216 people from the control group. They found a significant difference in *Bst*UI RFLP genotype frequency between ACL rupture and physically active controls among female participants but not among males. Participants' CC genotype was significantly underrepresented in the anterior cruciate ligament torn



group compared to controls (27.4% vs 5.6%; odds ratio = 6.6; 95% confidence interval, 1.5–29.7;  $p = 0.006$ ). There was no difference in the DpnII RFLP genotype distribution between the anterior cruciate ligament rupture groups and the physically active control groups [37].

O'Connell et al. [36] genotyped 333 patients with ACL injuries and 378 healthy controls (both South African and Polish) for COL3A1 rs1800255 (G/A), COL5A1 rs12722 (T/C), COL6A1 rs35796750 (T/C) and COL12A1 rs970547 (A/G). They found no significant associations between the COL6A1 rs35796750 and COL3A1 rs1800255 genotypes and ACL tear risk in the South African cohort; however, the COL3A1 AA genotype was significantly ( $P = 0.036$ ) overrepresented in the Polish ACL injury group compared to the control group [36].

Malila et al. [34] evaluated 86 patients with ACL tears and 100 healthy controls with no history of ligament/tendon injury for an association between the -1612 5A/6A polymorphism of the MMP3 gene and a predisposition to tearing the ACL. They observed that the frequencies of the 5A+ genotype (5A/5A, 5A/6A) and the 5A allele were significantly higher in contact sports players compared to non-contact sports players [34].

Mannion et al. [35] genotyped 227 ACL tear patients and 234 healthy controls for 10 polymorphisms in 5 genes encoding 5 proteoglycans. The proteoglycan molecules whose genes were tested were aggrecan – ACAN, biglycan – BGN, decorin – DCN, fibromodulin – FMOD, and lumican – LUM. Haplotypes were also constructed for specific regions. The authors observed that the G allele of ACAN rs1516797 was significantly underrepresented in the controls compared with the ACL group ( $P = .024$ ). For DCN rs516115, the GG genotype was overrepresented considerably in female controls ( $P = .015$ ) compared to the ACL group, and the AA genotype was significantly underrepresented in the control group ( $P = .013$ ) compared to a subgroup of women with non-contact ACL injuries. Haplotype analyses implicated regions overlapping ACAN (rs2351491 C>T-rs1042631 T>C-rs1516797 T>G), BGN (rs1126499 C>T-rs1042103 G>A), and LUM-DCN (rs2268578 T>C-rs13312816 A>T-rs516115 A>G) in the ACL tear predisposition. Based on the independent associations and the haplotype analysis, the authors postulated that regions within ACAN, BGN, and DCN and a region spanning LUM-DCN are positively associated with ACL tear susceptibility [35].

Khoury et al. [32] genotyped 141 ACL cases and 219 controls for the *ELN* rs2071307 and *FBN2* rs331079 variants. The *ELN* rs207137 variant was not associated with an ACL tear. However, the frequency of the G allele was significantly different between the ACL group (OR = 1.76 [95% CI, 1.00–3.10];  $P = .047$ ) and the controls. The DNA sequence variation within the *FBN2* gene is associated with ACL tears [32].

As can be seen, researchers analyze various genes in case-control studies and haplotype analyses. The results seem promising, but it should be emphasized that in this study it was noticed that the gender difference might be significant. As in the presented study – looking at gender, it can be observed that these significantly more frequent differences occur only in the group of men compared to the male control group (Table 1). Of course, one should remain critical of these results and consider it justified to research even larger groups, considering a larger number of tested genes.

## 5. Conclusions

Genetic research in sports, especially concerning injuries connected to characteristic polymorphic variants of selected genes, is justified, particularly on gender-homogenous groups.

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