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Multifactorial Analysis of Anterior Cruciate Ligament Injuries: Genetic Predisposition, Surgical Outcomes and Tunnel Widening

PhD thesis

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Table of Contents

List of Abbreviations....4
1 Introduction....6
1.1 Anatomy of the ACL....7
1.1.1 Origin....7
1.1.2 Insertion....8
1.1.3 Histology....9
1.2 Factors Predisposing to ACL Rupture....9
1.2.1 Intrinsic factors....10
1.2.1.1 Genetic factors....10
1.2.1.2 Age....13
1.2.1.3 Anatomy....13
1.2.1.3.1 Origin variations....13
1.2.1.3.2 Intercondylar notch width....14
1.2.1.3.3 Tibial anatomy....14
1.2.1.3.4 Joint laxity....14
1.2.1.4 Sex....15
1.2.1.5 Neuromuscular factors....16
1.2.2 Extrinsic factors....17
1.2.2.1 Floor....17
1.2.3 Sport discipline, and activity level....17
1.3 Risk Factors Associated with Graft Re-rupture....17
1.3.1 Surgery-related Factors....18
1.3.2 Graft choice....18
1.3.3 Age....19

1.3.4 Biological failure....20
1.3.5 Femoral tunnel formation....20
1.4 Tunnel enlargement....21
2 Objectives....24
2.1 Research Questions....25
3 Methods....26
3.1 Predisposing Factor: Genetic Analysis....26
3.1.1 Materials and methods....26
3.1.2 Statistical methods....27
3.2 Outcomes of ACL Reconstruction in Patients Under 16 Years....28
3.2.1 Materials and methods....28
3.2.1.1 Operation techniques....30
3.2.1.2 Rehabilitation....30
3.2.1.3 Statistical analysis....31
3.3 Possible Complications – Tunnel Widening....31
3.3.1 Material and methods....31
3.3.1.1 Rehabilitation....32
3.3.1.2 Statistical analysis....32
3.3.1.3 Radiological Assessment....33
4 Results....34
4.1 Predisposing Factor: Genetic Analysis....34
4.1.1 Study population demographics....34
4.1.2 Results of the Four-Group Patient Comparison....39
4.1.2.1 ACL - CON....39
4.1.2.2 ACL1 - CON....39
4.1.2.3 ACL2 - CON....39

4.1.2.4 ACL1 – ACL2....39
4.2 Outcomes of ACL Reconstruction in Patients Under 16 Years....40
4.2.1. Types of sports....42
4.2.2 Level of sports....45
4.2.3 Biological Sex....45
4.2.4. Comparison....46
4.3 Possible Complications – Tunnel Widening....48
5 Discussion....51
5.1 Predisposing Factor: Genetic Analysis....51
5.2 Outcomes of ACL Reconstruction in Patients Under 16 Years....54
5.3 Possible Complications – Tunnel Widening....57
5.4 Strengths and limitations....60
5.4.1 Genetic Analysis....60
5.4.2 ACLR under 16 Years old patients....60
5.4.3 Tunnel widening....61
5.5 Implication for practice and research....62
6 Conclusions....63
7 Summary....65
8 References....66
9 Bibliography of the candidate's publications....89
10 Acknowledgements....91

List of Abbreviations

ACAN Aggrecan
ACL Anterior Cruciate Ligament
ACLR Anterior Cruciate Ligament Reconstruction

ALL	Antero Lateral Ligament
AM	Anteromedial
ANOVA	Aanalysis Of Variance
BGN	Biglycan
COL	Collagens
DCN	Decorin,
ECM	Extracellular matrix
F	Female
FMOD	Fibromodulin
GSA	Global Screening Array
GWAS	Genome-Wide Association Study
HMS	Hypermobility Syndrome
HS	Hamstring
HSB	Handball, Soccer, Basketball
ICRS	International Cartilage Regeneration and Joint Preservation Society
IKDC	Knee Documentation Committee
IL	Interleukin
INHBA	Inhibin βA
KOOS	Knee Injury and Osteoarthritis Outcome Score
LD	Linkage Disequilibrium
LET	Lateral Extra-articular Tenodesis
LUM	Lumican
M	Male
MMPs	Matrix Metalloproteinases
MRI	Magnetic Resonance Imaging
OR	Odds Ratio
PL	Posterolateral
PT	Patellar Tendon
RNA	Ribonucleic Acid
ROM	Range of Motion
SNP	Single-Nucleotide Polymorphisms
TNF	Tumor Necrosis Factor

1 Introduction

The anterior cruciate ligament (ACL) injuries represent one of the most common and clinically significant musculoskeletal problems of our lifetime, particularly among physically active and athletic populations. The ACL rupture rate per 1,000 hours ranges from 0.005 to 0.2 across different team sports, and it is particularly common in team ball sports (1-6). Over recent decades, both their incidence and clinical relevance have steadily increased, which can be attributed partly to the growing intensity of sports activities and partly to advances in diagnostic and therapeutic methods (5, 7, 8). The ACL plays a crucial role in maintaining knee joint stability, especially in controlling anteroposterior translation and rotational movements; therefore, its' injury can lead to substantial functional impairment and long-term consequences.

In orthopaedic practice, ACL rupture represents a common injury that continuously raises clinically relevant questions concerning its underlying aetiology and the extent to which modifiable and non-modifiable factors contribute to its occurrence. ACL rupture should not be regarded solely as an acute traumatic event but rather as the result of a complex, multifactorial process in which biomechanical, anatomical, genetic and environmental factors all play a role. A significant proportion of these injuries occur through non-contact mechanisms, highlighting the importance of understanding predisposing factors in both prevention and treatment. Individual anatomical variations, differences in neuromuscular control, as well as hormonal and genetic influences, may all contribute to an increased risk of injury.

Conservative treatment often fails to provide satisfactory outcomes, particularly in young and active patients, where knee instability may lead to secondary damage such as meniscal injuries and early onset osteoarthritis (9-11). Consequently, ACL reconstruction has become a widely accepted and frequently performed surgical procedure. However, surgical management is not without complications. Graft re-rupture, insufficient biological integration and various technical factors may all influence surgical outcomes (12-15). Particular concern arises in younger patients, where a notably higher rate of re-injury can be observed. However, despite this clinical impression, the exact incidence within our own patient population, as well as its relation to published data, remains unclear. To evaluate whether advances in surgical techniques and rehabilitation strategies truly improve outcomes, it is essential to critically assess our own results in the context of the existing literature.

Furthermore, postoperative radiographic follow-up frequently reveals tunnel widening, a phenomenon that is often considered undesirable from a surgical perspective. Although literature remains inconclusive regarding its clinical relevance, reducing its occurrence may still be beneficial. This observation prompted us to investigate whether our department's routine use of double press-fit fixation for (bone-patellar tendon – bone) BTB ACL reconstruction—a standard, established technique—offers any specific advantages.

The present dissertation aims to provide a comprehensive overview of the factors involved in ACL injury, with particular emphasis on genetic background, the specific characteristics of ACL reconstruction in young patients and potential postoperative complications such as tunnel widening. The investigation of this topic requires a multidisciplinary approach, integrating clinical experience, imaging techniques and molecular biological research.

The significance of this research lies in improving the understanding of predisposing factors, thereby enabling the identification of high-risk groups, the development of preventive strategies and the establishment of personalized treatment protocols. Furthermore, it may contribute to the optimization of surgical techniques and the improvement of long-term outcomes. Overall, the study of ACL injuries is of paramount importance not only in orthopaedics and sports medicine but also in the broader context of preserving musculoskeletal health.

1.1 Anatomy of the ACL

1.1.1 Origin

The ACL originates with a crescent-shaped footprint spanning from the posterior aspect of the lateral intercondylar ridge (the resident’s ridge) to the posteroinferior surface of the lateral femoral condyle, extending directly to the boundary of the hyaline articular cartilage. At its femoral origin, the ligament fans out and measures approximately 11–16 mm in width and 2.5–3.8 mm in thickness. Although the anteromedial (AM) and posterolateral (PL) bundles are clearly defined at the tibial insertion, they cannot be macroscopically separated at the femoral origin (16). Histologically, the ligament’s fibres can be categorized into:

- Direct fibres, originating from the lateral intercondylar ridge and traversing the central core of the ligament.
- Indirect fibres, which spread in a ribbon-like configuration toward the posterior hyaline margin of the lateral femoral condyle.

The coordinated interplay between these fibre types ensures synchronized knee kinematics (17). Figure 1.

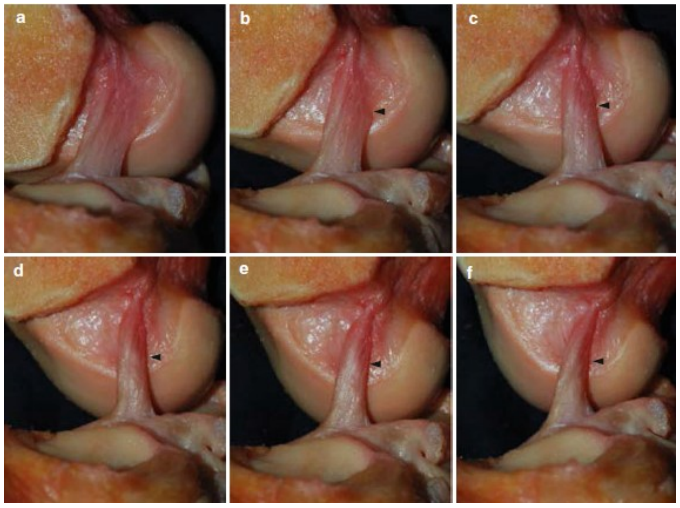


Figure 1. Dynamic observation of the midsubstance and fanlike extension fibers during flexion-extension motion of the knee (17). Under full knee extension, both fibre types remain taut. From approximately 15° of flexion onward, the sail-like indirect fibres begin to relax and, as flexion progresses, twist beneath the direct fibres (18,19).

1.1.2 Insertion

On the tibial side, the ACL inserts ventrally within the intercondylar region in a "C"-shaped footprint that encircles the medial intercondylar eminence. The ventral limb of the “C” corresponds to the AM bundle, whereas the dorsal limb represents the PL bundle. The total footprint area measures approximately 136 ± 33 mm², with an average width of 11 mm and depth of 17 mm. Minimal fibre insertions extend to the anterior and posterior horns of the lateral meniscus. These peripheral fibres, together with the meniscal attachments, form a tear-drop–shaped ring around the "C"-shaped footprint (20, 21). However, considerable anatomical variation exists. Beyond the classic “C” configuration, variants include “J”-shaped insertions or configurations resembling “Cc” corresponding to the two distinct bundles (17).

1.1.3 Histology

Duthon et al. examined the microscopic architecture of the ACL, showing it consists of fascicles measuring ~250 µm to 1–2 mm in diameter, predominantly composed of collagen fibres and enveloped by paratenon. These fascicles subdivide into 3–20 sub-fascicular bundles encased by an epitendineum and traversed by loose type II collagen. Sub-fascicular bundles contain fibres of 1–20 µm diameter, themselves composed of collagen fibrils (25–250 nm). Additionally, several collagen types are present in the ACL, with types I, II, III, IV, V, VI and XII being the most prevalent (22).

Histologically, the ACL is divided into three regions — proximal, middle and distal:

- Proximal region: Histologically heterogeneous, containing round to oval cells, spindle-shaped fibroblasts, collagen, and glycoproteins (fibronectin, laminin).
- Middle region: Predominantly spindle-shaped fibroblasts with abundant collagen. Its ventral surface comprises specialized fibrocartilaginous zones in contact with the femur during full knee extension. In addition to collagen, elastic fibres are present.
- Distal region: Characterized by decreased collagen fibre density, forming a compact structure composed of oval fibroblasts and chondroblast-like cells (22).

1.2 Factors Predisposing to ACL Rupture

Intrinsic and extrinsic factors may contribute to the development of ACL ruptures, therefore, understanding the biomechanical properties of the ligament is of particular importance. The tensile strength of the ACL is extremely high. In female athletes, Hewett et al. reported an average rupture load of 2,160 ± 150 N (23). Ordinary daily forces are insufficient to cause rupture; trauma, whether contact or non-contact, is required, with non-contact mechanisms accounting for approximately two-thirds of athletic ACL injuries (23). Tensile strength changes markedly with age, ranging from about 1,700 to 2,100 N in young individuals but dropping to roughly 650–730 N in older adults. A similar age-related decline is seen in ligament stiffness, which decreases from approximately 180–240 N/mm to around 130–180 N/mm over time (24, 25).

Additionally, repetitive microtraumas reduce tensile strength. Gallagher et al. demonstrated a decline in ACL strength with cumulative loading (26). Subcritical loads

may not rupture the ligament but can induce oedema. Csapo et al. reported up to a 40% increase in ligament volume during mid-season among skiers, which normalized by season's end, likely reflecting micro-damage and repair (27). This alteration, however, was resolved by the end of the season. The phenomenon described by the authors is explained by micro-injuries to the ligament and its' subsequent regeneration. Despite the very low number of cases, a similar curve was observed in all athletes. As long as this phenomenon does not occur too frequently, it is not a clinical concern, as the ligament can regenerate.

In contrast, Beaulieu et al. observed ACL and patellar tendon thickening in overloaded knees, underscoring the importance of collagen synthesis (28). However, a large number of repetitive microtraumas reduce the ligament's tensile strength, which may ultimately lead to rupture (28-31). According to Lipps and colleagues, ligaments ruptured earlier in the presence of higher-intensity repetitive trauma, and rupture also occurred more rapidly in the case of thinner ligaments (32). This may be one of the explanations for the higher incidence observed in female athletes (33). In addition to trauma, several well-known predisposing factors have also been identified.

1.2.1 Intrinsic factors

1.2.1.1 Genetic factors

A single nucleotide polymorphism (SNP) is the most common type of genetic variation in the human genome, involving a difference in a single nucleotide at a specific position in the DNA sequence. SNPs may influence gene expression, protein function, and individual susceptibility to diseases or injuries. In genetic association studies, SNPs are important biomarkers because they can help identify genetic variants linked to specific phenotypes, disease risks, or treatment outcomes. Therefore, the investigation of SNPs plays a crucial role in understanding the biological mechanisms underlying complex conditions and may contribute to the development of personalized medicine.

The role of genetic factors and specific gene polymorphisms in ACL rupture has not yet been clearly established; however, many patients report parental histories of ACL reconstruction, suggesting a potential hereditary predisposition. This raises the possibility that a defective gene may be heritable. Supporting this hypothesis, Flynn et al. found that

patients diagnosed with ACL rupture had a 2.0- to 2.2-fold higher likelihood of having a first-, second- or third-degree relative with an ACL tear than the general population (34). Similarly, Posthumus et al. reported significantly greater familial aggregation in patients with ACL injuries (35). Genetic studies related to ACL injuries can generally be divided into two main categories: those that focus on genes associated with connective and supporting tissues, and those that examine genes involved in tissue maturation and degradation processes (36).

It is a logical assumption that polymorphisms in genes encoding various types of collagen, may result in improperly structured collagen fibres (37). These defective fibres may, in turn, reduce the tensile strength of the ligament, thereby increasing the risk of rupture.

Early genetic research primarily focused on polymorphisms in collagen genes (35, 38-46). However, subsequent studies also investigated polymorphisms in other structural components such as fibrillin and elastin. Beyond structural proteins, several extracellular matrix (ECM) proteins have also been studied for their potential role in ligament integrity, including aggrecan (ACAN), biglycan (BGN), decorin (DCN), fibromodulin (FMOD) and lumican (LUM) (47-49). Nevertheless, the majority of these studies did not find a clear association between ACL rupture and polymorphisms in the specific gene segments examined, although the hit rate improved somewhat for proteoglycan-related genes. While a few statistically significant associations were identified, subsequent studies often failed to replicate these findings. By the late 2010s and into the 2020s, research interest shifted toward matrix metalloproteinases (MMPs), which play a key role in ECM remodelling, as well as genes involved in angiogenesis-associated signalling cascades (47, 50-54). Interestingly, these studies also produced similarly contradictory results. A summary of these findings is presented in Table 1.

Table 1. Frequency of significant and non-significant findings. The table indicates that studies finding no significant association outnumber those that did by nearly two to one.

Genes	No. of studies (significant)	No. of studies (not significant)
Collagens (COL)	8	10
COL1	4	3
COL3	2	2

COL5	1	2
COL6	0	1
COL12	1	2
Proteoglycans	7	8
ACAN	2	3
BGN	2	1
DCN	2	2
FMOD	0	1
LUM	1	1
ECM	0	2
Elasztin	0	1
Fibrillin	0	1
MMPs	1	9
MMP1	0	2
MMP3	1	2
MMP8	0	1
MMP10	0	2
MMP12	0	2
angiogenesis associated signalling cascade	1	2
VEGFA	1	1
COLIA	0	1
Summary	17	31

An interesting study by O'Connell et al. identified a significant correlation between the COL3A1 polymorphism and ACL rupture in the Polish population. In contrast, no such association was found in the South African population. The studies conducted so far are consistent in that they all utilize a candidate gene approach, focusing on polymorphisms in genes involved in ligament structure, ECM proteins or their maturation. In contrast, genome-wide studies have been published only in 2017 and 2021 (55, 56). These studies examined the entire gene region and focused on 98 candidate genes; however, statistical significance was found only for COL3A1. In the first study, no genome-wide significant associations were identified. Kim et al. reported borderline significance in three cases (rs113435565: 4.0×10^{-7}; rs11960097: 5.9×10^{-7}; rs4067493: 3.1×10^{-5}), although these were intergenic loci. In the second study, three SNP showed genome-wide significant associations with ACL and PCL injury, with p = 5 × 10-8 as the cutoff. rs144051132 is located on chromosome 7 in the 3' region of the Inhibin βA (INHBA) gene, which encodes a signaling molecule in the Transforming Growth

Factorbeta family that is a growth/ differentiation factor during development, including growth of osteoblastic cells involved in bone development. Rs186727643 is located on chromosome 12 in the 3' region of the Adipocyte Enhancer binding protein gene, which encodes a DNA-binding transcriptional repressor. Rs188099931 is located on chromosome 21 in the 3’ region of the long non-coding ribonucleic acid (RNA) gene (LOC101927869) of unknown function. The lack of significant findings among candidate genes in these studies is indeed surprising, as is the identification of genes reaching genome-wide significance. However, far-reaching conclusions cannot be drawn from these results. A major limitation of both studies is that they rely on existing genetic databases, in which the phenotypic characterization (i.e., the confirmed presence or definite absence of ACL rupture) is highly uncertain.

1.2.1.2 Age

The incidence of ACL rupture varies across different age groups. Most injuries occur between the ages of 15 and 30; however, a sex-specific distribution is evident. In the Nicholls study, the peak incidence in males occurred between the ages of 20 and 30. In contrast, a bimodal distribution was reported for females, with the highest incidence occurring around ages 16–17, followed by a marked decline in case numbers, and then a secondary, though more modest, peak in the late thirties (57).

1.2.1.3 Anatomy

1.2.1.3.1 Origin variations

It is a reasonable hypothesis that if the knee's anatomy deviates from normal, the joint's range of motion may be altered, potentially reducing the ligament's ability to resist mechanical forces. The opposite scenario is also possible, where the bony and cartilaginous structures of the knee appear within normal limits, but the orientation or trajectory of the ligaments differs. In an analysis of high-resolution volumetric magnetic resonance imaging (MRI) scans, Dimitriou et al. found that when the femoral origin of the ACL was located at least 30% more dorsally and 12% more distally relative to the normal axis of motion, the risk of ACL rupture was significantly higher, with an odds ratio (OR) of 51.2 (58).

1.2.1.3.2 Intercondylar notch width

Among the bony anatomical variations of the knee, the most frequently studied parameter is the morphological variation of the intercondylar notch. The ACL runs almost

entirely within the notch, making it a logical assumption, supported by several studies, that a narrower notch, particularly with a protruding lateral wall, may cause the ligament to lie against the bone, become impinged or alter its trajectory. Such changes may increase its susceptibility to injury (59-62). A narrower intercondylar notch and the resulting chronic irritation can reduce the structural integrity of the ACL.

1.2.1.3.3 Tibial anatomy

However, the femoral anatomy is not the only relevant factor. Bernhardson et al. found a linear correlation between the magnitude of forces acting on the ACL and the posterior tibial slope angle (63). An increased tibial slope increases the risk of ACL rupture in adults; however, this association has not been clearly established in populations under 18 years of age (64). In the tibia, not only the posterior slope of the plateau is relevant, but also the depth of the tibial plateau. A shallow medial tibial plateau may also increase the risk of ACL rupture (65).

1.2.1.3.4 Joint laxity

If ligaments are looser compared to the general population, the joint exhibits a greater range of motion. During movement, as ligaments are stretched, the bones gain greater kinetic energy, which, in turn, increases tensile forces within the ligaments, thereby increasing the risk of rupture. Joint laxity may be associated with generalized connective tissue disorders (e.g., Marfan syndrome, Ehlers-Danlos syndrome) or may present as an isolated condition, in which case it is referred to as hypermobility syndrome (HMS) (66, 67). The degree of laxity can be assessed using the Beighton score or the Brighton criteria (68). In daily clinical practice, the Beighton score is used due to its simplicity. Based on Csiák's work, the prevalence of HMS in the Hungarian population is 9.5% (69). In our study, we measured a significantly higher prevalence among patients undergoing ACL reconstruction at the Department of Sports Surgery at the National Institute of Sports Health. The prevalence of marked joint hyperlaxity was 24.6%, which was significantly higher than in the general population ($p < 0.001$) (70). Excessive knee joint laxity, however, may also confer certain advantages. In such cases, the compressive force binding the femur and tibia is significantly reduced, thereby decreasing the incidence of meniscal tears associated with ACL rupture (71).

1.2.1.4 Sex

Sex as a predisposing factor for ACL rupture has long been recognized; the authors found the injury to be 2 to 10 times more common among female athletes (72, 73). The exact cause is not fully understood. Potential contributing factors include neuromuscular differences, eccentric contraction of the quadriceps, anatomical variations such as a narrower intercondylar notch, more commonly seen in females, complex hormonal fluctuations, elevated estradiol level and differences in core musculature (74).

One of the primary functions of the ACL is to prevent anterior translation of the tibia. In approximately 30 degrees of knee flexion — typically occurring during sudden stops, rapid changes in direction or deceptive movements such as feints in ball sports — quadriceps contraction increases the anteriorly directed force on the tibia. During dynamic force measurements, tibial translation during muscle contraction was significantly lower in both male and female athletes than in untrained individuals. However, female athletes still performed significantly worse than their male counterparts. Furthermore, they were able to contract their hamstring muscles for a shorter duration than the male athletes (75).

The role of a narrower intercondylar notch has been discussed previously. Whether this anatomical variation occurs more frequently in females remains unclear. Several studies have addressed this topic, but no definitive association between notch width and sex has been found (76, 77).

Cyclical hormonal changes are characteristic of females. Several studies have demonstrated a correlation between the hormonal cycle and the incidence of ACL ligament tears (78, 79). Estradiol and progesterone receptors have been identified on the surface of the ACL in both humans and rats. In rat experiments, elevated concentrations of 17-β estradiol significantly reduced fibroblast proliferation and collagen synthesis in the ACL (80). Yu et al. also reported decreased fibroblast proliferation and type I collagen production, although their findings were based on fibroblast cultures derived from human ACL tissue (81). During ovulation, estradiol levels rise, coinciding with a decrease in ligament stiffness, thereby increasing its vulnerability to injury (79). Beyond female hormones, an intriguing question is whether the male hormone testosterone plays a role in ligament stiffness. Lovering et al. confirmed the presence of testosterone receptors on the

surface of the female ACL. Their study found a significant negative correlation between testosterone, free testosterone index and ligament stiffness during ovulation. Analysis of data from the Swedish ACL registry identified young female soccer players as the group most at risk for both recurrent and contralateral ACL ruptures (82).

1.2.1.5 Neuromuscular factors

The core musculature is the muscle group responsible for maintaining posture and proper trunk alignment, formed through the coordinated action of multiple muscles. It primarily includes the abdominal, back, hip, and pelvic muscles. During a bracing maneuver (e.g., a starting feint in handball), the player assumes a typical position in which the trunk leans forward and laterally in the direction opposite the feint, with the knees and hips flexed. Notably, the flexion angles at both joints are greater on the side corresponding to the direction of the feint (83). However, differences are observed between the sexes: in males, the trunk deviates farther forward, and the flexion angles at the hip and knee are greater Typically in males, maintaining this posture requires greater muscular strength, and the increased knee flexion offers enhanced protection against injury.Similar differences in landing movement have been described by Decker et al. (85). In their study, Decker et al. analyzed joint angles at landing, as well as the range of motion and angular velocities of these joints. Female participants exhibited greater knee and hip extension angles together with increased ankle plantarflexion at initial ground contact, leading to increased axial loading across the joints. At the end of the landing phase, women also exhibited greater peak flexion angles compared to men, which caused significantly higher joint angular velocities. Poor muscle balance and suboptimal energy absorption may also contribute to the increased risk of ACL injury in women (86). In Hewett's study, a significantly greater knee abduction angle was observed at landing in cases exhibiting dynamic valgus (intact vs. ACL-injured: 1.4° vs. 9°; $p < 0.01$), along with a significantly lower knee flexion angle (intact vs. ACL-injured: 82.4° vs. 71.9°; $p < 0.05$) and hip flexion angle (intact vs. ACL-injured: 74° vs. 33°; $p < 0.01$) (23).

1.2.2 Extrinsic factors

1.2.2.1 Floor

The relationship between ACL rupture and the playing surface has been relatively under-researched and sparsely published in the literature. We hypothesize that a higher surface friction coefficient - meaning the shoe grips more firmly - increases the risk of

ACL tears. In a 2003 study, Olsen et al. investigated the association between ligament injuries and synthetic flooring versus parquet surfaces. Analyzing 53 cases, they concluded that women had a 3 to 5 fold higher likelihood of sustaining an ACL rupture on synthetic surfaces. An interesting observation was that the coefficient of friction significantly depended on the shoe sole, showing up to a twofold difference, as well as *on the finish of the parquet and the cleaning agents used (87). Playing surface similarly influences injury incidence in American football. In Orchard's study, ankle injuries were 7 times more frequent on artificial turf, while knee injuries were significantly fewer on natural grass (88). An interesting aspect of the study is that, beyond the playing surface, environmental factors were also monitored. Cold and wet natural grass further reduced the incidence of injuries.

1.2.3 Sport discipline, and activity level

ACL ruptures most commonly occur during sports activities. The likelihood of rupture is naturally much higher in high-activity sports involving knee twisting, such as soccer, basketball, handball, volleyball and skiing (89-92). The level at which the athlete competes also matters. National team players and first-division athletes, who may train twice daily and compete in two to three matches per week, are exposed to a significantly higher risk (89, 93). It is well known that the number of injuries during matches is significantly higher than during training sessions (94).

1.3 **Risk Factors Associated with Graft Re-rupture**

Just as numerous predisposing factors for ACL rupture have been identified, a wide range of factors may also influence the outcome of surgical treatment. As with any surgical intervention, the outcomes of ACL reconstruction are not uniformly successful. Reported re-rupture rates show considerable variability, ranging from 2% to 25%. (95). Many of the factors that contribute to the initial ligament rupture also play a role in the development of re-rupture (e.g.: joint laxity, anatomy, sex, core muscle). In this chapter, I will discuss only those factors that have not yet been addressed, with one exception: the patient's age, which is examined to facilitate a better understanding of my analysis.

1.3.1 Surgery-related Factors

In cases of unsuccessful rehabilitation, graft re-rupture or a subsequent meniscal injury, the surgeon must always first verify the adequacy of the surgical technique.

Surgical intervention may be associated with several errors, including graft-harvesting errors, tunnel malposition and fixation difficulties (96, 97). A detailed discussion of these issues would far exceed the scope of this study. Since they are not directly related to the primary subject of my dissertation, further elaboration will be omitted. In the early years, efforts to restore knee stability focused mainly on ligament reconstruction, but over time, greater emphasis has been placed on the role of secondary stabilizing factors. Meniscus suturing basically reduces the development of osteoarthritis (98). In recent decades, root tears and ramp lesions have been described as having a significant secondary stabilizing role in the knee, therefore their management is critical (99). Various adjunctive lateral extra-articular procedures, such as LET and ALL reconstruction, have been increasingly adopted from the mid-2010s. The use of these techniques significantly reduces the risk of graft re-rupture, reoperations and improves knee function (100-103). In addition, graft choice, patient age and biological failure may contribute to re-injury.

1.3.2 Graft choice

It is logical to suggest that different grafts have varying susceptibilities to rupture. The tensile strength of the native ACL is approximately 2160 N (23). The tensile strength of various grafts is higher than this, making them suitable for replacement. There is no significant difference between the patellar tendon and the quadriceps tendon (patellar tendon: 2375 N, quadriceps tendon: 2352 N). However, the quadrupled hamstring graft used in our practice is substantially stronger (4108 N).

Consequently, the preferred graft has evolved over the years. In the 1990s, the patellar tendon was the predominant graft. One of the main advantages of this graft is that the longest follow-up data are available regarding its clinical outcomes. In addition, its use is not associated with flexion strength deficit, the healing occurs through bone-to-bone integration. However, this graft is also associated with the highest donor-site morbidity, with anterior knee pain and kneeling pain being common postoperative complaints. Furthermore, technical difficulties may arise during graft harvesting, including complications such as iatrogenic patellar fracture.

During the 2000s, hamstring tendon grafts became the preferred choice for ACL reconstruction. The advantages of this graft include the requirement for only a small surgical incision, low donor-site morbidity, and a relatively short operative time,

however, its disadvantages include reduced flexion strength and the possibility of slightly increased residual knee laxity.

Since the mid-2010s, the adoption of quadriceps tendon grafts has gradually increased (104, 105). The advantage of using a quadriceps tendon graft is that it results in lower anterior knee pain and kneeling pain, compared to the patellar tendon, it has low donor-site morbidity, and allows for the harvesting of either a full soft-tissue or a bone-block graft, however, this is the graft option for which we have the shortest follow-up data available.

Numerous studies have been published on graft re-ruptures across different graft types; however, large-sample meta-analyses do not show significant differences for autografts (patellar tendon: 2-6%, hamstring tendon: 4-10%, quadriceps tendon: 2-3%) (106-109). What is consistent in the literature is that, compared to autografts, allografts yield inferior outcomes, with a higher rate of re-injury (90, 110). The incidence of re-rupture is not determined solely by graft choice.

1.3.3 Age

It may seem logical to assume that children recover more effectively following ACL reconstruction; however, both clinical experience and the scientific literature indicate that this is unfortunately not the case. In younger age groups, particularly among adolescent athletes, the re-injury rate is significantly higher. Although the exact mechanism is not yet fully understood, several factors may contribute to this phenomenon.

On one hand, young patients are more likely to return to high-intensity, pivoting sports (e.g., football, handball, basketball), which place increased stress on the new graft and has an increased risk for a new injury as well (111). Another potential factor is the lack of fully developed neuromuscular control, as well as immature decision-making, which may lead to riskier behaviour in sports (112, 113).

The exact cause of the higher re-injury rate in young individuals is therefore multifactorial; nevertheless, younger age in itself is undoubtedly one of the most important objectively measurable predisposing factors. Reported re-injury rates vary widely in the literature, ranging from 2% to 25% (95). According to data from the

Swedish ligament registry, for example, Thorolfsson et al. reported a twofold higher graft rupture rate among patients under the age of 20 compared to older age groups (114). It has also been clearly demonstrated that returning to sports further increases the risk of graft rupture, particularly in high-intensity contact sports (111).

1.3.4 Biological failure

Biological failure also plays a role in ACL graft re-injuries, particularly when no surgical technical error or new trauma is present. Ménétrey emphasizes that early graft necrosis, insufficient revascularization, reduced cell proliferation, and lack of ligamentization may all contribute to failure, even when the graft is mechanically well positioned (115). This is further supported by a recent review, which demonstrated that the biological integration of allografts differs from that of autografts, thereby further increasing the risk of graft re-rupture (116).

1.3.5 Femoral tunnel formation

A thorough understanding of basic anatomy is essential for selecting the appropriate surgical technique. Historically, the femoral origin of the ACL was considered to be an oval area; today, a fan-shaped origin is widely accepted. The femoral tunnel was previously created almost exclusively through the transtibial portal. Based on updated anatomical knowledge, drilling through the AM portal is now the preferred technique. However, the literature remains inconclusive as to whether this approach decreases the rate of re-rupture or enhances surgical outcomes in terms of patient satisfaction (117-122). Another tibia-independent drilling technique is the so-called retrodrill. In a study by Tejwan involving 19,000 patients, both the AM portal and retrodrill techniques were associated with a higher likelihood of aseptic revision ACL reconstruction compared to the conventional transtibial technique (123). However, the use of the retrodrill can reduce the potential iatrogenic injuries associated with the AM portal technique (124).

1.4 Tunnel enlargement

Tunnel widening after ACL reconstruction is a well-known phenomenon. Its incidence ranges from 0 – 74% (125). The exact etiology of tunnel widening is still unknown. Tunnel widening is considered to develop within the first 6 weeks (126). This phenomenon is explained by the theory that Sharpey fibres, which contribute to tendon-to-

bone integration, develop within the first 6 weeks (127, 128). Based on CT examinations performed at the first and sixth postoperative weeks, as well as at 3, 6, 12 and 24 months, the degree of tunnel widening shows practically negligible change after 6 week (129). The widening is more pronounced with the hamstring graft than with the patellar tendon graft (130-133). In the case of hamstring tendon grafts, femoral widening is 20–40%, whereas with patellar tendon grafts, it is significantly smaller, approximately 16% (133-135). Amano et al. demonstrated that, in hamstring grafts, a greater degree of widening occurs (41.9% vs. 16%) with a different directional pattern (anterior and distal vs. distal only) (118-122). Besides the graft type, several factors can trigger it, such as movement of the graft within the tunnel, accelerated rehabilitation, the age of the patient, the size and the position of the drilled holes, the different types and devices of the graft fixation and higher cytokine activity (126, 136-142).

From rehabilitation perspective, two factors may influence tunnel widening: weight-bearing and mobilization. Using the same surgical technique, Tajima et al. found that, one year after surgery, when the non-weight-bearing period was reduced from 2 weeks to 1 week, greater widening was observed at the posterolateral femoral tunnel (143). Early mobilization increases the extent of tunnel widening (144, 145). If the tunnel wall has not yet undergone sclerosis—predominantly within the first 4 weeks after surgery—graft motion during mobilization can compress the cancellous bone, thereby contributing to tunnel enlargement (144). Wearing a brace in full extension for two weeks after surgery significantly reduces the degree of tunnel widening when hamstring tendons are used (146). Clinical results indicate that even at older ages, over 50 or even 60 years, ACL reconstruction can be performed in highly active athletes, provided there are no other contraindications, and the outcomes are comparable to those achieved in younger patients (147). Fewer studies have investigated whether patient age influences tunnel widening. In a 2016 study, Yanagisawa reported a significant correlation between femoral tunnel widening and patient age in double-bundle reconstruction: femoral tunnels were more enlarged in patients over 40 years of age (148).

It may increase or prolong the exposure of the adjacent bone to the synovial fluid if the graft is relatively flat in a round bone tunnel. This is referred to as the “synovial bathing effect” (142, 149). The tunnel-widening effect of synovial fluid infiltration is supported by the fact that, if the cruciate ligament stump is preserved, the graft achieves

better synovial membrane coverage. This coverage prevents synovial fluid from infiltrating the tunnel, thereby reducing tunnel widening (150, 151). If we reduce graft movement in the bone tunnel (windshield-wiper effect) with a fixation method (e.g., interference screw, press-fit technique) next to the joint line, we can decrease the synovial inflow. However, the most commonly used cannulated interference screw has a central hole and a space between the screw thread and the bone, both of which can allow synovial inflow. If we use the press-fit fixation technique, we can close the aperture of the femoral tunnel with the base of the bone block. With fixation closer to the joint, the potential motion of the graft within the tunnel is reduced, thereby decreasing the extent of tunnel widening (138). Biodegradable interference screws offer the benefit of not hindering revision procedures, yet their resorption can lead to cyst formation and greater tunnel enlargement (152). Conversely, polylactide or hydroxyapatite screws have been associated with less tibial tunnel enlargement than metallic screws (153).

A common theory is that synovial fluid enters the tunnel between the graft and the bone, leading to a series of disruptions in the normal bone-tendon healing process (126). After surgery, proinflammatory cytokine levels (Tumor Necrosis Factor (TNF)-α, Interleukin (IL)-1β, IL-6) in intra-articular fluid improve (142, 154). These cytokines stimulate osteoclast activity, contributing to bone resorption (155, 156). Due to graft movement, synovial fluid can leak into the tunnels (125). Inflammation can be either sterile or triggered by an infectious pathogen bacteria. When examining the latter, a clear correlation can be demonstrated during revision ACL reconstruction between the extent of tunnel widening and the presence of bacterial DNA (157).

The AM portal technique may reduce tunnel widening, but clinical results are inconsistent. Experimental attempts to mitigate widening have been made: autologous bone grafting showed a small, non-significant effect (3.8 mm vs. 3.5 mm), whereas the use of a fibrin mesh significantly reduced both femoral and tibial tunnel enlargement (158, 159). Tibial tunnels are also prone to widening, although the degree is generally less than that observed on the femoral side (135). An inverse relationship between tunnel diameter and later tunnel widening was reported by Sauer et al. (7 mm = +1.93 mm, 8 mm = +1.43 mm, 9 mm = +0.83 mm) (160). Tibial tunnel enlargement is similarly reduced when patellar tendon grafts are used (129, 133).

Other factors also play a role, including the length and position of the femoral tunnel, as well as the posterior slope of the tibial plateau (161-163). Femoral tunnels that are longer or situated more anteriorly tend to undergo greater widening, and those with a flatter orientation show a significant increase in tunnel enlargement.

The clinical significance remains uncertain: Webster found a positive correlation between tibial sagittal widening and anterior instability, whereas other studies have not demonstrated a clear relationship between tunnel widening and clinical outcomes (129, 132, 133, 164).

2 Objectives

The formulation of the present research questions was strongly influenced by everyday clinical experience. Recurrent observations during patient care, such as the high incidence of ACL injuries, the increased rate of re-rupture in younger individuals, and the frequent radiological finding of tunnel widening, highlighted several unresolved issues that warranted systematic investigation.

ACL injuries and their surgical management pose a significant challenge for athletes, particularly among young individuals and participants in high-risk sports. Several factors may contribute to ACL rupture, including anatomical and neuromuscular characteristics, as well as genetic predisposition. The aim of this thesis is to examine the risk factors and treatment options for ACL injuries from three different perspectives, with particular emphasis on genetic predispositions, management of patients across different age groups and the efficacy of surgical techniques.

First, the aim of this thesis is to explore the genetic background of ACL rupture. By examining the role of individual genes, we seek to identify gene variants that may predispose individuals to ACL injuries. Previous patient-based studies have primarily focused on candidate genes, referring to genes selected a priori based on their presumed involvement in ligament structure, extracellular matrix composition, or tissue remodelling processes relevant to ACL integrity; consequently, these studies were hypothesis-driven and typically investigated only one or a few specific SNPs at a time. In contrast, the present study adopts a hypothesis-free approach using genome-wide association study (GWAS) methodology, enabling the identification of novel genetic associations without prior assumptions. This technique scans the entire genome of a large cohort to identify variations, specifically hundreds of thousands of SNPs, associated with a particular trait or disease. By comparing the genetic profiles of affected patients against healthy controls, GWAS can pinpoint statistically significant genomic regions linked to injury susceptibility. The primary advantage of this approach is its unbiased nature, which allows for the discovery of previously unknown genes and biological pathways that traditional, targeted studies might completely overlook.

The second objective of this thesis is to examine the surgical outcomes in patients younger than 16 years and compare them with those of patients older than 16. Additionally, we aim to investigate how activity level and sex influence these outcomes.

The third objective of the study is to analyze tunnel widening, a common occurrence after ACL reconstruction, focusing on its incidence.

Summary:

1. Recruitment of suitable patients for the genetic study and establishment of an accurate database with reliably determined knee phenotypes.
2. Whole-genome analysis using the GWAS method.
3. Analysis of surgical outcomes in adolescent patients undergoing ACL reconstruction.
4. Comparison of surgical outcomes between patients under 16 years of age and those over 16 years of age.
5. Comparison of post-operative tunnel widening between patients with hamstring and patellar tendon grafts.

2.1 Research Questions

1. Which genetic polymorphisms contribute to the development of ACL rupture?
2. What are the outcomes of ACL reconstruction in patients younger than 16 years of age?
3. What is the impact of sex and athletic activity level on the surgical outcomes of ACL reconstruction?
4. Is there a difference in surgical outcomes between patients younger than 16 years and those older than 16 years?
5. What differences in tunnel widening are observed following ACL reconstruction using patellar versus hamstring tendon grafts, with particular attention to the incidence of femoral tunnel enlargement?

3 Methods

3.1 Predisposing Factor: Genetic Analysis

3.1.1 Materials and methods

This case–control prospective study was conducted between 2019 and 2024 at the National Institute of Sport Medicine Health (NISM), Semmelweis University Department of Molecular Biology, Hungarian University of Sports Science and the Pasamed Clinic in Budapest, Hungary.

Participants were consecutively recruited across multiple sites and classified into three distinct cohorts: patients undergoing arthroscopic unilateral ACL reconstruction (ACL1), those undergoing bilateral ACL reconstruction (ACL2), and a control group (CON). In cases where two separate reconstructions were performed on the same knee, patients were assigned to the ACL1 group. Regarding recruitment sites, ACL1 and ACL2 samples were collected during hospitalization for surgery at the NISM. Control samples were obtained from active athlete patients attending the Pasamed Clinic and students from the Hungarian University of Sports Science. Genetic analyses were performed blinded to all clinical outcomes to minimize measurement bias.

To ensure uniform eligibility, the inclusion criteria were strictly defined for each cohort. For the injury groups (ACL1 and ACL2), inclusion required a standardized diagnosis of ACL rupture verified by a four-pillar protocol: a documented history of knee trauma, positive clinical signs during physical examination (specifically the anterior drawer and Lachman tests), MRI demonstrating a clear disruption of the ligament, and final intraoperative verification during arthroscopy. Concomitant routine meniscal or cartilage interventions did not serve as reasons for exclusion and were not factored into group allocation. The control group comprised active athletes aged ≥19 years with no history of ACL injury or clinical signs of knee instability. Globally, all study participants across all cohorts were required to be of Hungarian origin with verified European ancestry to minimize population stratification and heterogeneity. Additionally, all participants provided written informed consent and yielded high-quality buccal DNA with a minimum concentration of 50 ng/μL.

Participants were excluded from the study or subsequent analyses if they withdrew their informed consent at any stage, or underwent concurrent major knee

procedures that confounded phenotype allocation. Furthermore, individuals with non-Hungarian citizenship or those whose parents were of non-Hungarian descent were excluded to maintain population homogeneity.

Although a formal statistical power analysis was not performed, sample size considerations were guided by two main factors. First, sample sizes from previous relevant studies were taken into account. Second, target numbers were determined in consultation with experts from the Department of Molecular Biology at Semmelweis University. Based on these considerations, and the hypothesis that bilateral ACL injury may reflect a stronger genetic predisposition, a cohort comprising at least 100 bilateral cases, 300 unilateral cases, and 200 controls was compiled to optimize statistical feasibility.

DNA was collected from buccal epithelial cells using duplicate swabs and stored at −20 °C until processing. DNA purification was performed at the Department of Molecular Biology, Semmelweis University, initiated with an overnight incubation of the samples at 56 °C in 0.2 mg/ml proteinase K cell lysis buffer. This was followed by denaturing the proteins with a saturated NaCl solution. Finally, the DNA was precipitated using isopropanol, followed by 70% ethanol. The DNA pellet was then resuspended in 100 µL of 0.5× TE buffer (0.005 M Tris-HCl, pH 8.0, and 0.5 mM EDTA). The concentration and quality of the DNA samples were assessed using a NanoDrop One spectrophotometer to measure the A260/280 and A260/230 ratios. All samples had a minimum DNA concentration of 50 ng/µL. The purified DNA-samples were stored at –20 °C until subsequent analysis.

The study was conducted in accordance with the Declaration of Helsinki and approved by the relevant institutional ethics committees (No.: 800-6/2019/EÜIG).

3.1.2 Statistical methods

Genotyping was performed using the Illumina Global Screening Array (GSA) MD v3.0. Raw intensity data (.idat files) were converted to genotype calls using bcftools plugins (+idat2g tc and +gtc2vcf) with manufacturer-provided cluster and manifest files (GSA-24v3-0_A1). Genotypes were aligned to the GRCh37/hg19 human reference genome and annotated using dbSNP rsIDs.

Quality control (QC) was performed using PLINK v1.9. SNPs and individuals with call rates below 80% were excluded, followed by a stricter 98% threshold. Individuals were further removed based on sex discrepancies, excess heterozygosity, or cryptic relatedness (PI_HAT > 0.2), retaining the individual with lower missingness within each related pair. Variants with a minor allele frequency (MAF) <1% or significant deviation from Hardy–Weinberg equilibrium ($p < 1\times10^{-6}$ in controls, $p < 1\times10^{-10}$ globally) were removed.

Only autosomal SNPs were retained. To identify heterozygosity outliers and related individuals, SNPs in long-range linkage disequilibrium (LD) regions were excluded, and LD pruning was applied to the remaining variants (50-SNP window, step size 5, $r^2 < 0.2$). The dataset was merged with the 1000 Genomes Project Phase 1 reference panel (20100804 release), strand-aligned, and filtered for mismatched SNPs. Multidimensional scaling was performed using pruned SNPs, and the top 10 multidimensional scaling components were used to infer genetic ancestry. Only individuals clustering with the European reference group were retained. Genome-wide association analyses were conducted using logistic regression in PLINK, assuming an additive genetic model and adjusting for the top 10 MDS components as covariates.

SNPs with missing or invalid test statistics were excluded from downstream analysis. A suggestive significance threshold of $p < 5\times10^{-5}$ was applied to identify candidate loci.

Comparisons between the study groups were performed using analysis of variance (ANOVA).

3.2 Outcomes of ACL Reconstruction in Patients Under 16 Years

3.2.1 Materials and methods

Patients who underwent primary ACL reconstruction at our institution due to a complete anterior cruciate ligament rupture were considered eligible for inclusion. The cohort consisted of athletes younger than 16 years and those older than 16 years at the time of surgery. Only patients who had participated in organized sports activities prior to injury and had a minimum postoperative follow-up of two years were included. Furthermore, inclusion required the availability of complete clinical documentation,

operative reports, and follow-up data. Patients treated with either hamstring tendon or bone–patellar tendon–bone autograft reconstruction techniques were eligible for analysis.

Unfortunately, precise documentation regarding the patients' preoperative sports activity was not available. The interval between injury and surgical reconstruction was typically 4–6 weeks. Acute surgical management was not performed. Instead, surgery was delayed until the knee joint had recovered from the acute inflammatory phase and full range of motion was restored, with the aim of reducing the risk of postoperative arthrofibrosis.

In our retrospective case-control study, we created two groups. In the first group, the patients underwent ACL reconstruction under the age of 16 years (Group 1); in the second group, the patients were older than 16 years at the time of ACL reconstruction (Group 2). The exclusion criteria were (1) multiple-ligament injuries and (2) ICRS grade III-IV. osteoarthritis at the time of ACL reconstruction. Concomitant meniscal injury was not an exclusion criterion.

As the first step, the patients completed our self-completion questionnaire, in which we assessed the value of the International Knee Documentation Committee (IKDC) subjective, Lysholm, Knee Injury and Osteoarthritis Outcome Score (KOOS), and Tegner scores, and we recorded all knee surgeries of the patients since their first ACL reconstruction. During the physical examination, we measured the Range of Motion (ROM), carried out Lachman, anterior drawer and pivot shift tests and assessed laxity with a KT-1000 arthrometer at 67 and 89 N on both knees. The tests were carried out by two separate orthopedic surgeons. In cases where discrepancies between the measurements were observed, the patients were re-evaluated jointly, and a consensus measurement was established.

In the second group (Group 2), we analyzed the Lysholm score, KOOS, Tegner Activity Scale, IKDC scores, as well as the number and types of knee surgeries.

For comparability, the data were standardized to a 4-year follow-up time to match the second group (Group 2).

Patients in both groups were contacted twice by telephone to take part in the study.

All procedures performed in our studies involving human participants were in accordance with the ethical standards of the institutional and national research committees and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the regional ethical committee (No. 15/2019).

3.2.1.1 Operation techniques

In patients who underwent ACL reconstruction with hamstring tendons, quadrupled semitendinosus and gracilis tendon grafts were used with an Endobutton suspensory fixation system at the femoral and two spiked staples at the tibial side. The operation technique was precisely described by Kawaguchi et al. (165).

The remaining patients underwent ACL reconstruction with a bone–patellar tendon–bone graft. We used an implant-free, double press-fit fixation technique for graft fixation (166, 167). In surgeries performed with both grafts, the femoral tunnel was created via the transtibial technique. In the case of open growth zones, whenever significant growth was still expected and no other associated injuries were present, we tried to postpone the ACL reconstruction until the onset of closure. In these cases, then a fixation type bridging the zone (such as an interference screw) was not used. We used a patellar tendon graft when the growth plates had already closed.

3.2.1.2 Rehabilitation

The patients had the same rehabilitation protocol. The operated knee was fixed at 0° in a postoperative brace. However, during daily physiotherapy sessions, the brace was removed and the knee was mobilized within a 0–40° range to gradually restore range of motion. We initiated isometric muscle strengthening exercises immediately after the surgery. In the first week, full non-weight bearing was applied. We started a full range of movement at the 4th postoperative week. The patients could begin cycling at six weeks, swimming at ten weeks, and running in a straight line at twelve weeks after the operation. Sport-specific exercises were started at week 16. We allowed the patients to return to sports within the ninth postoperative month.

3.2.1.3 Statistical analysis

We compared the number of surgeries, the types and levels of sports, as well as the sex distribution via the two-sample Z test, whereas the analysis of patient-reported outcomes was carried out via two-sample t tests. The level of significance was set at $p<0.05$, and the analysis was performed in R software (version: 4.2.2).

3.3 Possible Complications – Tunnel Widening

3.3.1 Material and methods

In the third part of my thesis, we analyzed tunnel widening following ACL reconstruction. This retrospective study was conducted on 467 patients with ACL surgery in our sports surgery department between 2003 and 2015. Inclusion criteria were (1) age above 13 years, (2) availability of standardized postoperative AP and lateral radiographs obtained 6 months after surgery and (3) adherence to the same postoperative rehabilitation protocol. Exclusion criteria were (1) history of previous ACL reconstruction of either knee, (2) multiple ligament injury, (3) evidence of osteoarthritis on radiographs. The concomitant meniscal injury was not an exclusion criterion. Two groups were created according to the graft used, the hamstring (HS) group and the patellar-tendon (PT) group. The operations were performed in the HS group with quadrupled semitendinosus and gracilis tendon graft using a suspensory fixation system with endobutton at the femoral and 2 spiked-staples at the tibial end. The single-bundle reconstruction was precisely described by Kawaguchi et al (165).

The bone-patellar tendon-bone graft was harvested with the technique in Pavlik et al (167). The most important step of this technique was to very accurately shape the patellar bone. It had a trapezoid form with most commonly 9mm diameter at the end and 10mm diameter at the base. In this case, the patellar bone block was impacted into the 9mm wide femoral tunnel. The femoral tunnel and the patellar bone must have the same length, hence the base of the patellar bone fit totally into the femoral tunnel, without any fixation device. A tibial fixation was performed using a similarly shaped trapezoidal bone block.

All of the operations were performed by one senior surgeon (TH). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964

Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the regional ethical committee. (No. 16/2019).

3.3.1.1 Rehabilitation

The patients had the same rehabilitation protocol. After the surgery, they were wearing a 0° fixed brace for three weeks. The patients practiced isometric muscle stretching and 30-40° flexion during this time. We allowed full weight-bearing one week after the operation. On the fourth postoperative week, full range of movement was initiated. Bicycling was allowed after 6 weeks, swimming after 10 weeks, and straight-line running after 12 weeks. Sport-specific exercises were started in week 16. We allowed the return to sport at the ninth postoperative month.

3.3.1.2 Statistical analysis

Comparisons between groups with regards to age and operation time were performed using two-sample t-tests. The ratio of the tunnel widenings was compared using a one-sample t-test. The difference in the tunnel widenings between the groups was compared using a two-sample t-test. We used the free R software for the statistical analysis. (R version 3.6.3. The R Foundation for Statistical Computing) The significance level was set at $p = 0.05$.

3.3.1.3 Radiological Assessment

Standard anteroposterior (AP) and lateral view X-rays were performed at six months postoperatively. Figure 2. The femoral and tibial tunnel widenings were measured 1 cm far from the aperture of the tunnels, perpendicular to the axis of the tunnels. We defined it as the distances between the two sclerotic bone margins. The comparison was made between the measured value and the drilled tunnels**.**

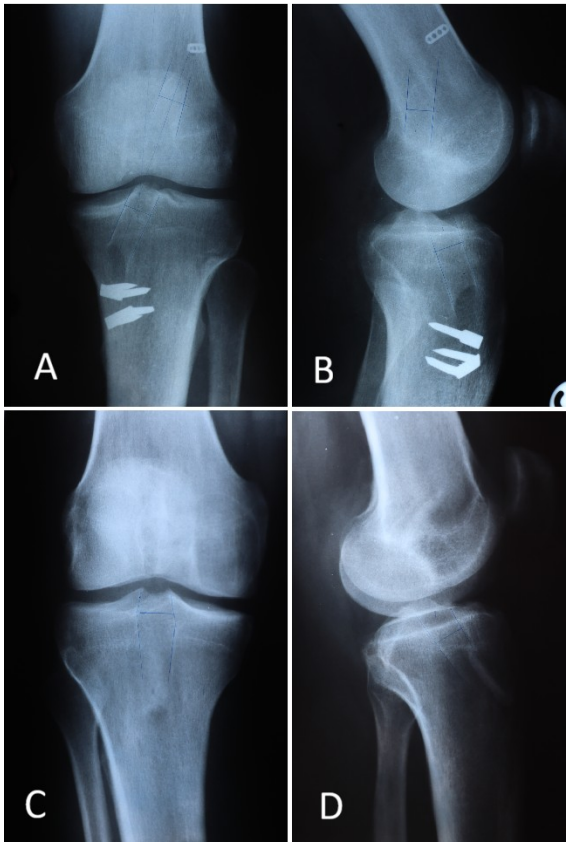


Figure 2. Six-month follow-up radiographs. Anteroposterior (A) and lateral (B) views following hamstring tendon reconstruction, and anteroposterior (C) and lateral (D) views following patellar tendon reconstruction.

4 Results

4.1 Predisposing Factor: Genetic Analysis

4.1.1 Study population demographics

A total of 647 individuals were included in the genetic study. All individuals who were invited to participate in the study agreed to enroll, reflecting a high participation rate. This may be partly attributed to the strong patient-physician relationship characteristic of the Hungarian healthcare system. All participants have signed written informed consent. Only one participant withdrew consent during the study without providing a specific reason.

During DNA extraction, our sample size decreased by 17. In some cases, the samples were taken from closely related individuals. Due to the higher genetic similarity of these participants, 23 individuals were excluded from the analysis. So we ultimately analyzed 607 samples. The ACL1 group included 293 patients, the ACL2 group 108, and the control group 206; the sex distribution of the groups is shown in Table 2.

No significant differences were observed between the groups in sex distribution. (χ^2 test, P = 0.5789). However, ANOVA demonstrated significant between-group differences in patient age and Tegner activity score (Age: P=0.00432; Tegner score: P=0.0124)

Table 2. Sample demographics. SD: standard deviation; No significant differences were observed in sex distribution, while age was significantly lower in the ACL1 group compared with controls, and Tegner scores were significantly lower in both ACL groups compared with controls

Groups	Sex (Male/Female)	Mean Age (Years ± SD)	Mean Tegner Score ± SD
Control (CON)	112:94 (54%: 46%)	33.3 ± 12.1	7.84 ± 1.6
Unilateral ACL rupture (ACL1)	173:120 (59%: 41%)	30.2 ± 11.4	7.42 ± 1.7
Bilateral ACL rupture (ACL2)	61:47 (56%: 44%)	33.9 ± 11.2	7.4 ± 1.8

The results are shown in the Table 3.

Table 3. List of SNPs reaching suggestive significance ($p < 5 \times 10^{-5}$). Legend: SNP: single nucleotide polymorphism; EA: effect allele; OR: odds ratio; CI: confidence interval. Clustering of variants was observed in GALNT11 and ITGAM (ACL–CON), HSPA12A (ACL1–CON), OPRD1 (ACL2–CON), and NEK10 (ACL1–ACL2), while no genome-wide significant associations were identified.

ACL – CON							ACL1 - CON						
SNP	**EA**	**OR**	**CI95_L**	**CI95_U**	**P value**	**Gene**	**SNP**	**EA**	**OR**	**CI95_L**	**CI95_U**	**P value**	**Gene**
rs916570	G	1.857	1.381	2.498	4.231e-05	-	rs1597170	C	0.5081	0.3742	0.6898	1.427e-05	-
rs1264695	G	1.933	1.42	2.63	2.771e-05	-	rs12105936	A	0.4068	0.2652	0.6238	3.739e-05	**ANTXR1**
rs1108052	C	1.933	1.42	2.63	2.771e-05	-	rs1561002	C	1.99	1.438	2.754	3.331e-05	**NPAS2**
rs2240071	G	1.972	1.431	2.718	3.337e-05	-	rs11652004 7	A	0.1249	0.04796	0.3251	2.034e-05	-
rs1110446	T	1.996	1.45	2.748	2.252e-05	-	rs6836062	A	1.957	1.421	2.695	3.929e-05	-
rs36105131	T	0.16	0.06831	0.375	2.465e-05	**GALNT11**	rs6960113	G	0.5744	0.4422	0.7461	3.258e-05	**DOCK4**
rs7810799	A	0.1597	0.06813	0.3742	2.415e-05	**GALNT11**	rs73668362	A	4.628	2.403	8.916	4.645e-06	**DIPK1B**
rs28793074	G	0.1179	0.04586	0.3034	9.21e-06	**GALNT11**	rs1900500	C	0.5444	0.4151	0.7139	1.105e-05	**HSPA12A**
rs6944002	C	0.1358	0.05556	0.332	1.2e-05	**GALNT11**	rs2420330	A	1.896	1.393	2.582	4.837e-05	**HSPA12A**

SNP	EA	OR	CI95_L	CI95_U	P value	Gene	SNP	EA	OR	CI95_L	CI95_U	P value	Gene
rs7797214	T	0.13 58	0.05556	0.332	1.2e-05	**GALNT11**	rs2272485	A	2.13 9	1.49	3.071	3.728e-05	**OR7E47P**
rs73668362	A	3.93 1	2.069	7.469	2.913e-05	**DIPK1B**	rs6580850	G	2.15 2	1.499	3.088	3.219e-05	**OR7E47P**
rs3010455	T	1.75 6	1.362	2.263	1.397e-05	**C10orf82**	rs57406257	A	0.23 02	0.1167	0.4541	2.267e-05	-
rs1900500	C	0.56 72	0.4407	0.7302	1.078e-05	**C10orf82**	rs7305771	C	0.23 02	0.1167	0.4541	2.267e-05	-
rs2272485	A	2.14 7	1.523	3.028	1.3e-05	**OR7E47P**	rs7308829	C	0.22 3	0.1109	0.4485	2.552e-05	-
rs6580850	G	2.15 9	1.532	3.044	1.109e-05	**OR7E47P**	rs9543662	C	0.55 06	0.4231	0.7165	8.915e-06	-
rs35688572	C	4.64 6	2.269	9.513	2.659e-05	**NAV3**	rs12928810	A	0.42 82	0.2852	0.6428	4.275e-05	**ITGAM**
rs9543662	C	0.56 22	0.4394	0.7193	4.635e-06	-	rs10492855	T	0.10 69	0.03981	0.2871	9.153e-06	**C16orf87**
rs1954074	G	0.51 63	0.3766	0.7078	4.008e-05	**LOC**	rs2341648	G	0.30 77	0.1775	0.5334	2.691e-05	-
rs12928810	A	0.46 56	0.3234	0.6703	3.936e-05	**ITGAM**	rs4967703	G	0.26 73	0.1464	0.4882	1.753e-05	-
rs9888739	T	0.46 77	0.3247	0.6738	4.514e-05	**ITGAM**	rs7203206	C	0.26 73	0.1464	0.4882	1.753e-05	-
rs11860650	T	0.46 95	0.3264	0.6752	4.529e-05	**ITGAM**	rs9939872	T	0.25 28	0.1364	0.4684	1.246e-05	-
rs10492855	T	0.21 15	0.1073	0.417	7.285e-06	**C16orf87**	rs8060193	G	0.26 6	0.1457	0.4858	1.636e-05	-
rs4967703	G	0.33 1	0.1993	0.5499	1.958e-05	-	rs4966800	C	0.26 85	0.147	0.4901	1.853e-05	-

SNP	EA	OR	CI95_L	CI95_U	P value	Gene
rs7203206	C	0.331	0.1993	0.5499	1.958e-05	-
rs9939872	T	0.3211	0.1921	0.5365	1.441e-05	-
rs8060193	G	0.3294	0.1983	0.5473	1.812e-05	-
rs4966800	C	0.332	0.1999	0.5515	2.055e-05	-
rs72802536	T	0.1338	0.05209	0.3436	2.921e-05	LOC

SNP	EA	OR	CI95_L	CI95_U	P value	Gene
rs10418701	C	0.5208	0.3809	0.712	4.358e-05	-

ACL2 – CON

SNP	EA	OR	CI95_L	CI95_U	P value	Gene
rs6669447	C	2.326	1.564	3.459	3.055e-05	OPRD1
rs2236857	C	2.434	1.598	3.706	3.392e-05	OPRD1
rs2298896	G	2.295	1.543	3.412	4.079e-05	OPRD1
rs12749204	G	2.571	1.65	4.007	2.99e-05	OPRD1
rs640493	G	0.46	0.3174	0.6666	4.086e-05	TNNI3K
rs7548905	A	3.359	1.928	5.852	1.875e-05	VAV3

ACL1 - ACL2

SNP	EA	OR	CI95_L	CI95_U	P value	Gene
rs9789461	A	3.321	1.94	5.685	1.202e-05	-
rs10203445	C	2.837	1.729	4.655	3.69e-05	-
rs711578	C	2.046	1.452	2.884	4.288e-05	LRRN1
rs78078666	T	3.541	2.203	5.69	1.749e-07	-
rs78633931	A	2.867	1.823	4.509	5.165e-06	-
rs2100006	G	0.4915	0.3509	0.6883	3.57e-05	NEK10

SNP	EA	OR	CI95_L	CI95_U	P value	Gene	SNP	EA	OR	CI95_L	CI95_U	P value	Gene
rs1528424	C	0.3795	0.2385	0.6039	4.365e-05	ALK	rs552647	C	0.498	0.3557	0.6972	4.891e-05	NEK10
rs1919987	G	0.4438	0.3016	0.6531	3.778e-05	CPNE4	rs583298	T	0.498	0.3557	0.6972	4.891e-05	NEK10
rs350655	A	3.257	1.852	5.727	4.118e-05	LINC	rs4973768	T	2.109	1.51	2.948	1.23e-05	NEK10
rs7022160	G	2.319	1.567	3.434	2.626e-05	LOC	rs11652004 7	A	9.983	3.545	28.12	1.33e-05	-
rs12342183	A	2.255	1.536	3.311	3.338e-05	LOC	rs79307194	T	4.076	2.132	7.794	2.141e-05	LOC
rs11801467 2	A	5.625	2.557	12.37	1.749e-05	-	rs2289230	G	0.4745	0.3355	0.6711	2.5e-05	CPE
rs35688572	C	5.956	2.606	13.62	2.337e-05	-	rs30656	A	0.3521	0.2148	0.5772	3.485e-05	ADAMTS 19
rs7024	T	2.218	1.549	3.174	1.352e-05	SEMA4B, CIB1	rs75692009	G	2.299	1.55	3.412	3.54e-05	-
rs2154571	G	2.887	1.783	4.674	1.616e-05	LOC	rs11724393 0	A	6.542	2.83	15.12	1.113e-05	ENTPD1
rs78995019	T	4.395	2.266	8.526	1.188e-05	-	rs7130530	C	0.3264	0.1928	0.5526	3.064e-05	CADM1
rs2833192	T	4.395	2.266	8.526	1.188e-05	LOC	rs78995019	T	3.375	1.878	6.066	4.778e-05	-
rs9985134	G	4.741	2.413	9.316	6.305e-06	-	rs2833192	T	3.375	1.878	6.066	4.778e-05	LOC

For polymorphisms with p-values exceeding 0.001, the observed significance values ranged between 5×10^{-5} and 5×10^{-7}

4.1.2 Results of the Four-Group Patient Comparison

4.1.2.1 ACL - CON

The most robust finding was the GALNT11 gene, around which 5 SNPs were identified. We found 3 SNPs associated with the ITGAM gene, 2 SNPs each for the C10orf82 and OR7E47P genes, and 1-1 SNP for the DIPK1B, NAV3, and C16orf87 genes.

4.1.2.2 ACL1 - CON

When comparing the unilateral ACL rupture group and the control group, 2 SNPs each were identified in the HSPA12A and OR7E47P genes. Additionally, 1 SNP was detected in each of the following genes: ANTXR1, NPAS2, DOCK4, DIPK1B, ITGAM, and C16orf87. The significance levels in these cases also ranged between 5×10^{-5} and 10^{-6}.

4.1.2.3 ACL2 - CON

In this group, 4 SNPs were identified in a single gene, OPRD1. In all other cases, 1 SNP each was detected in the TNNI3K, VAV3, ALK, CPNE4, LINC, SEMA4B, and CIB1 genes. All significance values were in the order of 10^{-5}.

4.1.2.4 ACL1 – ACL2

In this group, 4 SNPs were identified around a single gene, NEK10. The remaining findings involved only 1 SNP each for the LRRN1, CPE, ADAMTS19, ENTPD1, and CADM1 genes, with significance values in the range 10^{-5}. The significance levels of the results were visualized using Manhattan plots. Figure 3.

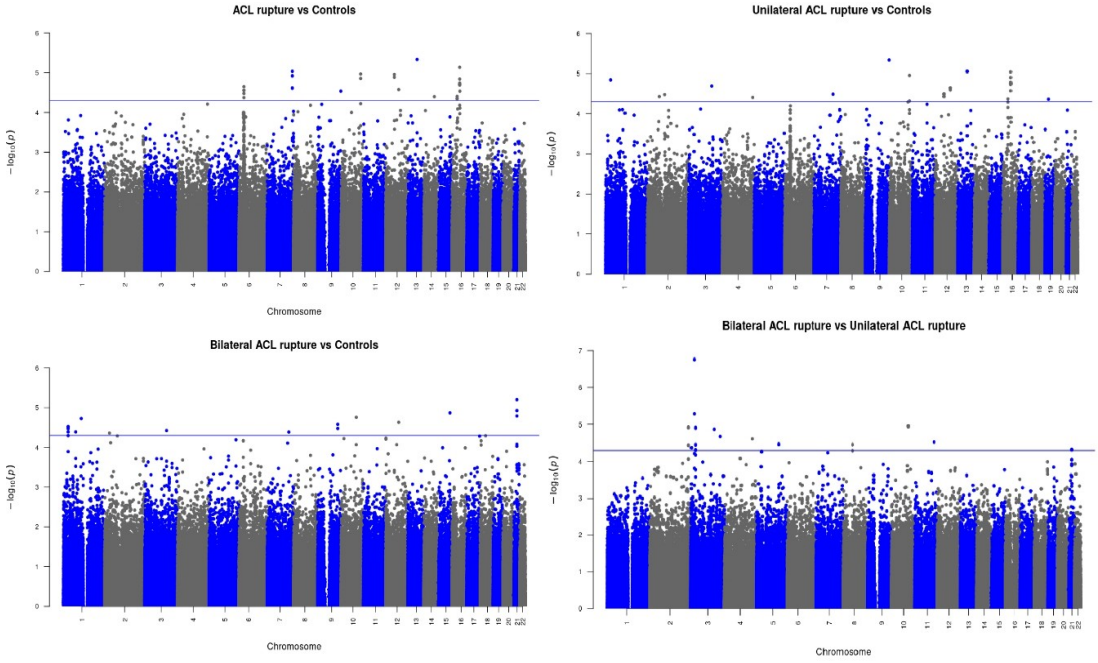


Figure 3. Manhattan plots. Manhattan plots for the investigated ACL rupture phenotypes, with the strongest association signal observed in the comparison of ACL1 vs. ACL2 cases.

The statistical analysis did not reveal any genome wide significant association ($P<5\times10^{-8}$). (Table 3.)

4.2 Outcomes of ACL Reconstruction in Patients Under 16 Years

There were 289 ACL reconstructions among athletes under 16 years of age (Group 1) between 2007 and 2022 in our Department of Sports Surgery. Owing to multiple changes in hospital software, we only had 194 telephone numbers or email addresses. Since the patients were minors at the time of ACL reconstruction, we only had access to their parents' contact information. In several cases, the patients' contact details were not provided, so we were able to reach 154 patients. In total, we included 70 patients (45%) who underwent clinical examinations during follow-up. All 70 patients included completed the questionnaire in full.

The second group (Group 2) consisted of 143 patients who underwent ACL reconstruction between 2016 and 2020, all of whom were over 16 years old at the time of surgery. In this group, an online survey was conducted. Altogether, 86 athletes completed our questionnaire (60%).

We found an improvement in the number of ACL surgeries per year in patients under 16 years of age. Figure 4.

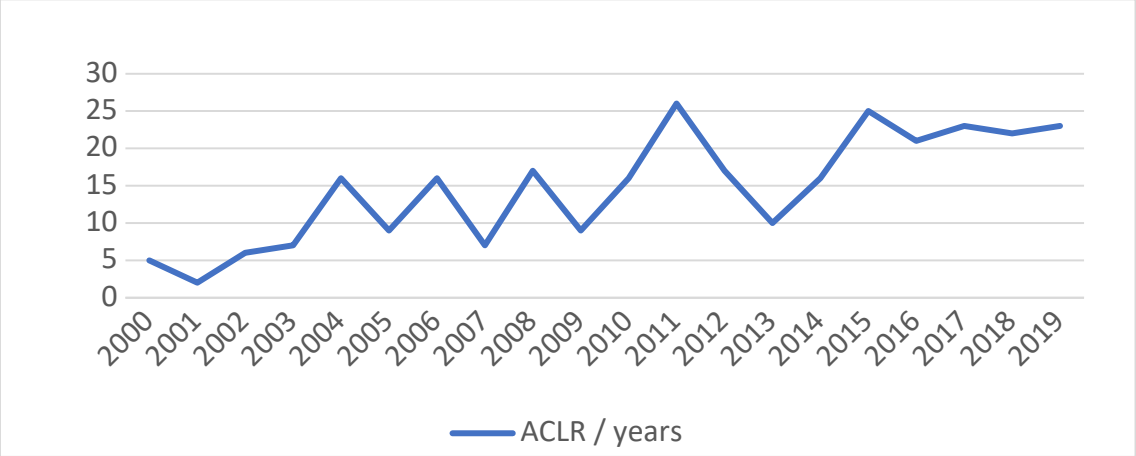
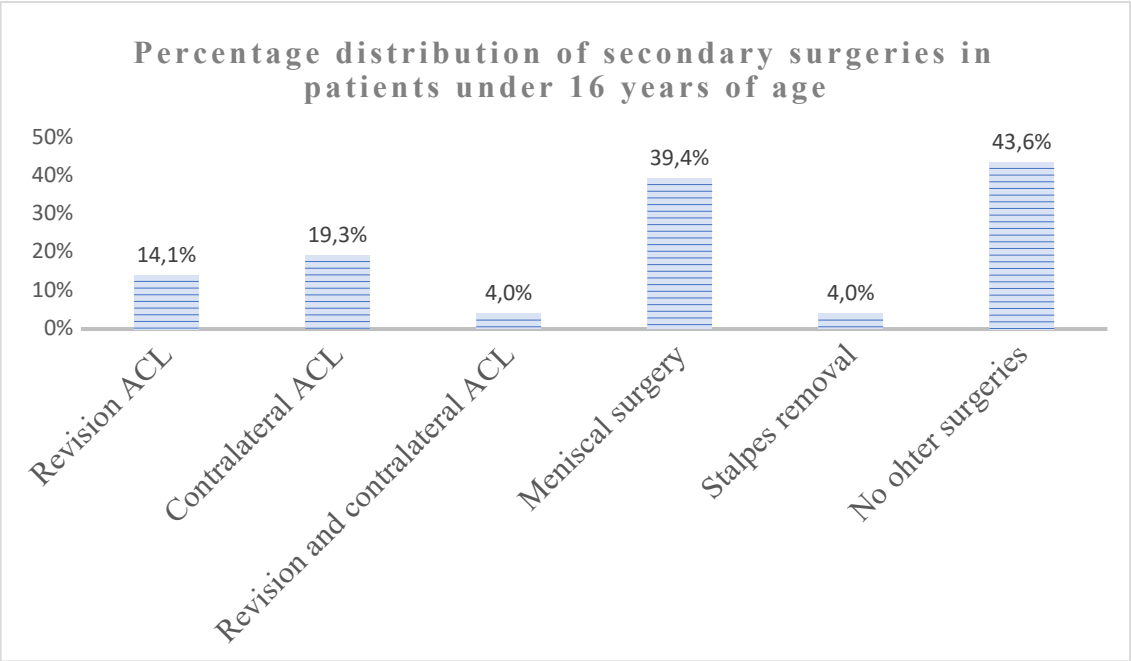


Figure 4. Anterior Cruciate Ligament Reconstruction (ACLR) numbers between 2000 and 2020 The number of operations rose significantly, from 5-6 procedures in the early 2000s to 20-25 procedures in the late 2010s.

There were 22 males and 48 females (M/F:22/48; 31%/69%) with a mean chronological age of 15.2 (13.2–15.99 years) years. Sixty-five patients underwent ACL reconstruction with hamstring grafts (semitendinosus and gracilis), and 5 patients with patellar tendon grafts. The mean follow-up time was 6.9 years (range: 2–19.3 years), and the mean age at the time of follow-up was 22.1 years (range: 15.2–35.2 years).

Among patients under the age of 16 years, 10 patients underwent revision ACL reconstruction (14.1%), and 13 patients underwent ACL reconstruction on the contralateral side (18.3%). The median time of the revision ACL reconstruction was 28 months (7–66 months), whereas it was 53 months (12–138 months) for the contralateral side. Among the revisions, 70% occurred in the first three years after ACL reconstruction. Three patients (4%) underwent both revision and contralateral ACL reconstruction. A total of 28 meniscal surgeries were required in 22 patients (39.4% of the surgeries, representing 31% of the patients). In three cases, it was necessary to remove the staples (4%). Only 31 patients (43.6%) did not undergo any secondary surgeries. Figure 5.

Figure 5. Percentage distribution of secondary surgeries in patients under 16 years of age. Additional knee surgical intervention was performed in 56.4% of the patients.



Fifty-three patients (75%) had an average side-to-side difference of 1.9 mm with the KT-1000 arthrometer at 89 N. Regarding ROM, 19 patients (27%) had an average of 4 degrees less extension, whereas 36 patients (51%) had an average of 9 degrees less flexion.

The patient-reported outcome results were as follows: mean KOOS score 86.7 (48.4–100); IKDC subjective score 87.1 (54–100); and Lysholm score 89.5 (43–100). The results of the KOOS subgroup were as follows: KOOS Pain: 96.0 (75–100); KOOS symptoms: 76.5 (50–100); KOOS Daily: 98.9 (86.8–100); KOOS Sport: 88.4 (10–100); and KOOS QOL: 73.3 (6.25–100). The median Tegner score was 8.0 before and 7.0 after ACL reconstruction.

4.2.1. Types of sports

Of all participants, 74% were athletes in either handball (22 athletes, 31.4%), soccer (17 athletes, 24.3%), or basketball (13 athletes, 18.6%). For simplicity, these sports are grouped under the abbreviation HSB (handball, soccer, basketball). Additionally, one athlete from each of the following sports participated: alpine skiing, acrobatics, riding, fencing, triathlon, orienteering, water polo, dancing, badminton, artistic gymnastics, athletics, karate, and volleyball.

Table 4. Demographic data of the Groups. Demographic and sports characteristics of two groups, including age, follow-up time, sex distribution, sports participation level, and types of sports practiced.

Groups	**Group 1**	**Group 2**	
Number	70	86	
Sex (F/M)	48/22	33/53	
Age (Years)	15,2	30,8	
Follow-up (years)	6.9	3.66	
Level of sports (C/R)	60/10	35/48	
No sport		3	
Type of Sports:			
handball	22	31	**soccer**
soccer	17	11	**handball**
basketball	14	7	**alpine skiing, bicycle**
Volleyball	3	3	**basketball, athletics, artistic gymnastics**
athletics, karate/combat sport	2	5	**dancing**
alpine skiing, fencing, water polo, triathlon, orienteering, dancing, riding, badminton, artistic gymnastics, acrobatics	1	2	**volleyball, karate/combat sport, ice hockey, wrestling**
		1	**badminton, aerobic, rowing, snowboard, auto-motor sports**

The percentage of revision was 15% among the three dominant sport types, i.e., team athletes, whereas the revision rate was only 11% among athletes of other sports. The difference was not statistically significant (P=0.326). A similar pattern was observed in contralateral ACL reconstructions, with a rate of 21% in team athletes compared to 11% in others, although this difference was also not statistically significant (P = 0.173).

Table 5. Analysis of Revision ACLR and Contralateral Rates According to Sport Type, Activity Level, and Sex. This table presents the distribution of patients who underwent revision ACLR and contralateral ACLR based on type of sport, level of sports activity, and sex. Revision ACLR – No / Yes: Indicates the number and percentage of patients who did not (No) or did (Yes) undergo revision ACL reconstruction. Contralateral ACLR – No / Yes: Indicates the number and percentage of patients who did not (No) or did (Yes) undergo ACL reconstruction on the opposite knee. Other: Refers to patients participating in sports other than HSB sports. Recreational: Patients who play sports casually or for leisure. Competitive: Patients who participate in organized sports at a competitive level. Male / Female: Biological sex of the patients.

	Revision ACLR numbers				**Contralateral ACL Rnumber**		
revision ACLR	No	Yes	Total	**contralateral ACLR**	No	Yes	Total
Other	16(89%)	2(11%)	18(100%)	**Other**	16(89%)	2(11%)	18(100%)
HSB	44(85%)	8(15%)	52(100%)	**HSB**	41(79%)	11(21%)	52(100%)
Total	60	10	70	**Total**	57	13	70
		P=0.326				*P*=0.173	
revision ACLR	No	Yes	Total	**contralateral ACLR**	No	Yes	
Recreational	9(90%)	1(10%)	10(100%)	**Recreational**	16(89%)	2(11%)	18(100%)
Competitive	51(85%)	9(15%)	60(100%)	**Competitive**	49(82%)	11(18%)	60(100%)
Total	60	10	70	**Total**	57	13	70
		P= 0.338				P= 0.236	

	Revision ACLR numbers				Contralateral ACLR number		
Revision ACLR	No	Yes	Total	**Contralateral ACLR**	No	Yes	Total
Male	20(91%)	2(9%)	22(100%)	**Male**	21(95%)	1(5%)	22(100%)
Female	40(83%)	8(17%)	48(100%)	**Female**	36(75%)	12(25%)	48(100%)
Total	60	10	70	**Total**	60	10	70
		P=0.200				P=0.020	

Each subgroup shows the number of patients and the percentage (in parentheses) relative to the total in that subgroup. P-values are provided to indicate statistical significance of differences between subgroups. A P-value less than 0.05 is considered statistically significant.

4.2.2 Level of sports

Most athletes played sports at a high level; 60 (86%) played competitive sports, and only 10 patients (14%) played recreational sports. There was no statistically significant difference in the proportion of competitive and recreational sports within the revision group (90%) and the contralateral ACL reconstruction group (85%). (revision: P= 0.338; contralateral: P=0.236). (Table 3.)

4.2.3 Biological Sex

Eight of the athletes who underwent revision ACL reconstruction were female (80%), whereas the rate was even higher among those who underwent contralateral ACL reconstruction (females: 12 patients 92%; males: 1 patient 8%). The proportion of females was significantly greater in the case of contralateral ACL reconstruction (P=0.020), whereas for revision, the difference was not statistically significant (P=0.200). (Table 3.)

4.2.4. Comparison

Compared with Group 1, the sex distribution ratio was the opposite (M53:F33). The mean age at ACL reconstruction was 30.8 (16.1–51.5 years) years, and the mean follow-up time was 3.66 (2.1–6.8 years) years. All 86 patients included completed the questionnaire in full.

In the second group (Group 2), 15 patients needed a further surgical procedure in the operated knee following ACL reconstruction (17%).

We found five revision ACL surgeries (5.8%) in Group 2. Among these five patients, three were under 20 years of age. The mean age of the five patients was 21.9 years. Among the procedures performed following ACL reconstruction, six arthroscopic arthrolysis procedures (6.9%), four meniscus resections (4.6%), and one (1%) meniscal refixation, microfracture, and metal removal procedure were performed. Furthermore, 11 patients underwent multiple operations on the ipsilateral side (12%).

On the contralateral side, four patients (4.6%) required surgery: one ACL reconstruction (1%), two meniscus resections (2.3%) and one meniscus refixation (1%).

The average age of the two groups showed a significant difference (Group 1: 14.8 years vs. Group 2: 30.8 years; $p<0.0001$).

A comparison of the groups is shown in Table 6.

Table 6. Comparison of the groups. This table summarizes the baseline characteristics of the two study groups based on age: Group 1 includes patients younger than 16 years, and Group 2 includes patients older than 16 years at the time of surgery. The table shows the number of patients in each group, mean follow-up time (in years), sex distribution (female:male), mean age, and the average Tegner activity level before injury.

Groups	Group 1 (x<16)	Group 2 (x>16)
Number	45	86
Follow-up (x<8years)	3.72	3.66
Sex (F/M)	33:12	33:53
Age (Years)	14.8	30.8
Tegner before	8.0	7.8

The number of meniscus surgeries (Group 1: 26% vs. Group 2: 4.6%; p=0.003), contralateral ACL reconstruction (Group 1: 8.8% vs. Group 2: 1.1%; P=0.03) and total number of surgeries (Group 1: 44% vs. Group 2: 21%; p=0.01) were significantly greater among patients under 16 years of age. We also found several cases of revision ACL reconstruction in Group 1, although the difference was not significant (Group 1: 11% vs. Group 2: 5.8%; p=0.293).

Table 7. Comparison of the groups based on the number of surgeries. This table compares the surgical outcomes between Group 1 (patients under 16 years) and Group 2 (patients over 16 years), such as revision ACL reconstruction (p=0.293), meniscus surgery (p=0.003), contralateral ACL reconstruction (p=0.03), and total number of surgeries (p=0.01), with statistically significant differences observed in all except revision ACL reconstruction.

Groups	**Group 1 (x<16)**	**Group 2 (x>16)**	**p score**
Revision ACLR	5/45 (11%)	5/86 (5.8%)	0.293
Meniscus surgery	12/45 (26%)	4/86 (4.6%)	**0.003**
Contralateral ACLR	4/45 (8.8%)	1/86 (1.1%)	**0.03**
Total number of surgeries	20/45 (44%)	18/86 (21%)	**0.01**

The Lysholm scores (Group 1: 88.3 vs. Group 2: 87.0; p=0.271), IKDC subjective scores (Group 1: 88.4 vs. Group 2: 86.9; p=0.478), mean KOOS scores (Group 1: 86.9 vs. Group 2: 86.5; p=0.498), KOOS QOL scores (Group 1: 75.4 vs. Group 2: 67.4; p=0.064), daily scores (Group 1: 98.6 vs. Group 2: 98.8; p=0.453), and sport scores (Group 1: 89.6 vs. Group 2: 87.3; p=0.324) did not significantly differ, whereas the KOOS symptom scores (Group 1: 75.6 vs. Group 2: 85.0; p<0.005) were significantly lower in Group 1. However, the KOOS pain subscale score was significantly lower in patients over 16 years of age (Group 1: 95.6 vs. Group 2: 94.0; p=0.033).

Table 8. Comparison of the groups on the basis of patient-reported outcomes. Comparison of postoperative outcome scores between patients under 16 and those 16 and older. Statistically significant differences were found in KOOS Pain (p=0.033) and KOOS Symptoms (p<0.005).

4.3 Possible

	Under 16 years	16 years and older	P score
IKDC subj.	88.4	86,9	0.478
Lysholm	88.3	87.0	0.271
Mean KOOS	86.9	86.5	0.498
Pain	95.6	94.0	**0.033**
Sympt.	75.6	85.0	**0.005**
Daily	98.6	98.8	0.453
Sport	89.6	87,3	0.324
QOL	75.4	67.4	0.064

Complications – Tunnel Widening

In the HS group, X-ray images of 248 patients were evaluated, while in the PT group, images of 219 patients were analyzed. There was no significant difference between the average age of the groups. However, the operation time was significantly longer in the PT group than in the HS group (HS:44 min; PT 53: min; p<0.001; 95%CI -11.53 - -7.02). In both groups, there were more males than females, and the difference between the groups was significant (HS: M:F 168:80; PT: M:F 180:39; p=0.0005).

Table 9. There was no significant difference between the average age of the groups. However, the operation time was significantly longer in the PT group than in the HS group (HS: 44 min; PT 53: min; p<0.001; 95% CI -11.53 - -7.02). In both groups, there were more males than females, and the difference between the groups was significant (HS: M:F 168:80; PT: M:F 180:39; p=0.0005).

	Hamstring (HS *n*=247)	**Patellar tendon (PT *n*=219)**	**p score**	**95% CI**
Age	27.4 years (13.5 – 57.2)	27,9 years (14.5 – 51.7)	0.528	-378.62 – 737.18
Sex	M:F 168:80	M:F 180:39	**0.0005**	n.a.

Operation time (min)	44 min (25 – 100)	53 min (30 – 100)	**<0.001**	-11.53 -7.02

The mean intraoperative tunnel diameter was at the femoral and tibial ends 7.4 mm (6–10 mm) in the HS group and 9.98 mm (9–12 mm) at the femoral, and 8.9 mm (8–10 mm) at the tibial end in the PT group.

The mean incidence rate of tunnel widening was, in the HS group, on the AP and the lateral views, 88% (n=217 and 83% (n=205) at the femoral end, and 97% (n=240) and 97% (n=240) at the tibial end. The incidence rate of tunnel widening, in the PT group was 17% (n=37) and 2% (n = 4) on the femoral AP and lateral views, and 94% (n=205) and 84% (n=184) on the tibial views.

There was a significant difference in the incidence rate of femoral tunnel widenings both on AP and lateral radiographs (HS vs. PT: fem. AP: 89% vs. 17% $p<0.001$; HS vs. PT: fem. lat: 84% vs. 2% $p<0.001$). On the tibial radiographs, a higher widening rate could be observed in the HS group, although the difference was significant only on the lateral images. (HS vs. PT: tib. AP: 97% vs. 94% $p= 0.163$; HS vs. PT tib. lat: 97% vs. 84% $p<0.001$).

Table 10. Comparison of femoral and tibial tunnel widening incidence rates between graft types. Tunnel widening was measured on radiographs at follow-up. HS: hamstring tendon graft; PT: patellar tendon graft. Values are expressed as mean ± standard deviation. Statistical comparisons between groups were performed using two-sample t-tests. Statistical significance was assessed using t-tests, with a significance level of $p < 0.05$.

	HS (n=248)	**PT (n=219)**	**p**
Femoral AP	88% (n=217)	17% (n=37)	**<0,001**
Femoral lateral	83% (n=205)	2% (n=4)	**<0,001**
Tibial AP	97% (n=240)	94% (n=205)	0,163
Tibial lateral	97% (n=240)	84% (n=184)	**<0,001**

Table 11. Magnitude of the tunnel widening by graft type. Except for the lateral femoral radiograph in the PT group, all of the tunnel widenings were significant. (p<0.001)

Graft	X-ray	Widening	p
HS (248)	**Femoral AP**	50% (4.15 mm)	**<0,001**
	Femoral lateral	47% (4.18 mm)	**<0,001**
	Tibial AP	38% (2.86 mm)	**<0,001**
	Tibial lateral	41% (3.04 mm)	**<0,001**
PT (219)	**Femoral AP**	39% (3.48 mm)	**<0,001**
	Femoral lateral	25% (2.2 mm)	n.a.
	Tibial AP	38% (3.8 mm)	**<0,001**
	Tibial lateral	32% (3.19 mm)	**<0,001**

The mean tunnel widening in the HS group was, on the femoral AP and lateral views, 4.15 mm (50%) and 4.18 mm (47%), and 2.86 mm (38%) and 3.04 mm (41%) on the tibial views. The mean tunnel widening in the PT group was 3.48 mm (39%) and 2.2 mm (25%) on the femoral AP and the lateral views, and 3.8 mm (38%) and 3.19 mm (32%) on the tibial views.

Because the incidence rate was low in the PT group on the femoral lateral X-ray, the statistical analysis was not possible in all cases. With this in mind, the tunnel widening on all views was significantly less in the PT group, except for the above-mentioned radiograph (fem. AP: 50–6% p<0.001 95% CI 0.40—inf; fem. lat: 47–0.5% p<0.001 95% CI 0,44—inf.; tib. AP 38–36% p =0.9 0.05—inf; tib. lat: 41–28% p<0.001 95% CI 0.09–0.17)

5 Discussion

5.1 Predisposing Factor: Genetic Analysis

ACL injury is common among athletes and often results in prolonged rehabilitation and substantial time loss. Injury risk is influenced by multiple external and internal factors, including neuromuscular, anatomical and potentially genetic determinants. This study investigated the association between ACL rupture and genetic variants using a hypothesis-free genome-wide approach in our cohort. Our findings suggest that polymorphisms in less studied regulatory genes may contribute to ligament vulnerability.

The most important result of our study is that we identified a possible genetic link between polymorphisms in the GALNT11, ITGAM, C10orf82, OR7E47P, HSPA12A, OPRD1 and NEK10 genes and ACL rupture. This study is the first to demonstrate the involvement of these genes.

Furthermore, this GWAS was conducted in our own well-characterised cohort, allowing accurate verification of the knee status of all included athletes. Another strength is that the control group consisted of active athletes rather than members of the general population.

However, we did not find genome-wide significant associations with ACL injury using $p = 5 \times 10^{-8}$ as a cut-off, but possible genetic link with significance levels below 5×10^{-5}.

When comparing all ACL rupture cases to controls (ACL–CON), our findings highlight several genes that may be involved in ligament vulnerability. Given that the ACL is primarily composed of type I and III collagen fibres, alterations in collagen remodelling may influence ligament tensile properties (37). In the present study, the strongest association was observed with polymorphisms in the ITGAM gene. ITGAM encodes the integrin αM (CD11b) subunit, which is involved in immune regulation and inflammatory responses, particularly through leukocyte adhesion and migration (168). Variants of ITGAM have previously been linked to systemic inflammatory conditions, including systemic lupus erythematosus (169), and to knee osteoarthritis, supporting its potential relevance in joint tissue pathology (170).

While several intrinsic and extrinsic factors contribute to ACL injury risk (3, 5–10), ligament rupture occurs when mechanical loading exceeds tensile capacity (171). During athletic activity, repetitive submaximal loading is common and may result in cumulative micro-injuries rather than immediate rupture (27). Imaging studies have demonstrated transient, load-related changes in ACL structure, suggesting adaptive remodelling processes following micro-damage (28). However, repeated microtrauma may impair tissue integrity, reduce tensile strength and increase rupture susceptibility (26, 28-31). Experimental data indicate that increased loading frequency and reduced ligament thickness accelerate failure, which may partially explain the higher incidence of ACL injuries observed in female athletes (32, 33).

The role of the ITGAM gene in predisposition to ACL rupture is not yet known. It is possible, in theory, that polymorphisms in this gene may play a role in defective injury–remodelling processes, which could lead to a decrease in the tensile strength of the ligament.

The GALNT11 gene encodes an enzyme involved in mucin-type O-glycosylation and regulates key signalling pathways, including notch signalling, with additional roles in cilia-related processes and left–right body asymmetry (172). It also influences receptor function, such as megalin (LRP2), and normal renal physiology (173). Dysfunction of GALNT11 has been associated with developmental abnormalities, including heterotaxy (174), although its role in cruciate ligament pathology has not previously been described.

Two SNPs were identified in the C10orf82 gene, which is thought to encode a microtubule-associated protein involved in ciliary and flagellar structure and stability; however, its precise biological function remains unclear (175). To date, no association between C10orf82 and ACL injury has been reported.

OR7E47P is a pseudogene that does not encode a functional olfactory receptor and has no known direct role in olfaction (176).

Focusing specifically on unilateral ACL ruptures compared with controls (ACL1–CON), additional gene associations were identified that may reflect mechanisms related to single-ligament injury. In the ACL1 group, we also identified 2 SNPs around a gene. One of the genes is the previously mentioned OR7E47P, while the other is HSPA12A.

This gene is an atypical member of the heat shock protein family. It plays a role in adaptation to metabolic and mechanical stress, and several of its functions are known. In their study, Mao et al. reported that in the heart it inhibits fibroblast activation, thereby preventing the development of fibrosis (177). It enhances angiogenesis and endothelial cell responses both in vitro and in vivo (178). Yu et al. emphasize in their study that it is essential for maintaining aerobic glycolysis and protein lactylation, which may also influence cellular stress responses and regenerative processes (179). To date, no studies have directly investigated its role in cruciate ligament injuries; however, it may be involved in the inflammatory processes accompanying micro-injury and tissue remodelling.

In the comparison of bilateral ACL ruptures and controls (ACL2–CON), a distinct pattern of genetic associations emerged, suggesting potentially different underlying biological mechanisms. Within this group, we observed enrichment of four SNPs at two distinct loci. One locus is located in an uncharacterized genomic region, while the other corresponds to the OPRD1 gene, which encodes a delta opioid receptor. Variants of OPRD1 have been linked to opioid dependence, modulate opioid-mediated signalling pathways, and influence pain perception (180). To date, no studies have directly investigated its role in cruciate ligament injuries.

Finally, when comparing unilateral and bilateral ACL rupture cases (ACL1–ACL2), additional differences in genetic variants were observed, suggesting factors contributing to increased susceptibility to repeated injury. We also identified an enrichment of four SNPs around the NEK10 gene. NEK10 is a versatile kinase involved in multiple critical biological processes, including cell cycle regulation, DNA damage responses, mitochondrial homeostasis, and ciliogenesis. Clinically, it has been associated with ciliopathies and potentially with cancer, and it may also influence cellular stress signaling and energy metabolism (181, 182).

A clear connection between ciliary function and ACL function has not been established to date. However, in our study, it is noteworthy that among the genes with multiple SNP hits, three (GALNT11, C10orf82, NEK10) are involved in the regulation of ciliary function or its development.

In addition, we identified two SNPs in an uncharacterized genomic region.

5.2 Outcomes of ACL Reconstruction in Patients Under 16 Years

The most important findings of this study were that the rates of contralateral ACL reconstruction (P=0.03), meniscal surgery (P=0.003), and the total number of operations (P=0.01) were significantly higher in patients under 16 years of age compared to those over 16 years. Although ACL revision rates also appeared higher in the younger group, this difference did not reach statistical significance (P=0.293).

The question arises whether the lower contralateral rupture and re-rupture rates in the patients over 16 years could be due to the shorter follow-up period. As the years progress, the occurrence of repeat injuries naturally increases, but we do observe that re-injuries are most common in the first 2-3 years following ACL reconstruction (13, 111). In our own study, graft re-rupture occurred on average 28 months after ACL reconstruction. Among the 13 cases of contralateral ACL rupture, the injury occurred within 3.6 years after ACL reconstruction in 6 cases, and later than 3.6 years in 7 cases. Given that the vast majority of re-ruptures occur within the first 36 months, we would have observed a higher rate in Group 2 even within the shorter 44-month follow-up period. We believe that the shorter follow-up period does not have a significant impact on the higher rate of revision injuries; however, to eliminate this potential source of bias, we standardized the follow-up period.

During the last three decades, there has been a high increase in the incidence of adolescent ACL rupture (183). The growing number of young athletes may be responsible for the rise in injuries, but early sports specialization and greater public and physician awareness of these injuries may also play a role (8, 184). One other possible reason for the higher rate of ACL graft re-rupture observed in young individuals is the presence of generalized joint hypermobility. This condition is characterized by an inherently more lax ligamentous system, which provides reduced joint stability and may increase the risk of graft failure under physical stress (185). Return to sport is a well-known risk factor, and an early return to pivoting sports in particular significantly increases the risk of re-injury (186). Compared with adult patients (83%), skeletally immature athletes have a greater return to sport level (92%), which may further increase the risk of graft re-rupture (187-190).

In our experience, the number of ACL reconstructions has steadily increased between 2000 (5 operations/year) and 2019 (23 operations/year), reflecting a growing trend in surgical treatment among adolescents.

One well-known and important risk factor for ACL graft rupture is previous ACL reconstruction, therefore, it is legitimate to question whether we should perform the reconstruction in the case of an anterior cruciate ligament tear (15). When an adult patient does not experience any symptoms or a locking sensation and does not participate in pivoting sports, conservative treatment may be considered an option (191). In contrast, nonoperative ACL rupture care has explicitly negative outcomes among adolescents; therefore, we should aim for the earliest possible surgical treatment (10, 11, 192-194). Nevertheless, not only the revision rates among adolescents are high, ranging from 9-35% but the contralateral ACL reconstruction rates (13-20%) as well (12, 195-201). The surgical results may be affected by the method of graft fixation, which is why Calvo's study is interesting, as he reported similar revision ACL reconstruction rates with the same surgical technique (202). Calvo et al. used the end button suspensory system with double-staple tibial fixation and reported more than 5 mm tibial translation in 22% of cases with respect to the healthy knee, although there was no statistically significant difference in functional scores between these patients and those with a difference of <5 mm. However, in our study, the rate of tibia translation greater than 5 mm was only 2.8%. Despite the difference in tibial translation, the revision rate was nearly the same (our study: 14.5% vs Calvo: 14,8%).

The double press-fit fixation technique used in patellar tendon reconstruction is relatively uncommon. Pavlik et al. investigated the stability of press-fit fixation in cadaver knees and found that, at a 60-degree femoral tunnel angle, graft rupture or bony block fracture was more likely to occur than fixation failure. Compared with the literature, these results demonstrated biomechanical properties similar to and satisfactory for femoral press-fit fixation (203). In Hidas's study, histological analysis revealed adequate ossification by six weeks following the press-fit technique (204). A major advantage of double press-fit fixation is that, in addition to providing satisfactory clinical outcomes, it eliminates the need for implant placement (167). As a result, the bony block achieves more than 90% integration on the femoral side, which significantly facilitates the placement of the femoral tunnel in potential revision surgeries (205).

In our sample, 28 patients (39.4%) required meniscus surgery following ACL reconstruction with a mean follow-up of 6.9 years. At a follow-up of 3.66 years, the rate of meniscal injuries was lower, at only 26%, indicating that the rate of meniscal injuries increased over time. However, patients older than 16 years had a significantly lower rate of meniscal tears (*P*=0.003). Fewer data are available on the rate of meniscal tears following ACL reconstruction than on revision rates. Chan's study reported that 10.7% of meniscal tears occurred after ACL reconstruction, whereas Ichinohe reported that only 4% did (206, 207). Unfortunately, in our study, we found a higher rate in patients under 16 years of age than in those over 16 years of age at both 3.66 and 6.9 years of average follow-up. For patients older than 16 years, the rate was 4.6%, which is in line with the literature.

Among patients who have been followed up, there are twice as many females as males (females: 48, males: 22). Nicholls et al. reported that among the adolescent population, ACL ruptures affect females the most (57). HMS is a predictive factor for revision and contralateral ACL surgeries and is also more common among females (208). This may be due to the high number of females in both our groups (revision: 80%, contralateral: 92%). The proportion of females was significantly greater in the case of contralateral ACL reconstruction (*P*=0.020), whereas for revision, the difference was not statistically significant (P=0.200). Ahldén et al. also reported that young female athletes have a significantly greater incidence of contralateral ACL ruptures when analyzing data from the Swedish Ligament Register (82).

Wiggins et al. reported that returning to sports increases the rate of re-injury (12). Moreover, it also makes a difference in which sport the athlete returns to. Athletes who play high-pivoting sports (e.g., football) have a greater risk of ACL re-rupture (199, 209). In contrast to ice hockey and basketball, Boch reported higher rates of re-injury among handball and football players (6). We hypothesized that the rates of revision and contralateral ACL reconstruction would be greater among competitive athletes performing high-pivoting ball sports. We could not prove our theory. The rates of revision and contralateral cruciate ligament reconstruction were not significantly higher among either competitive (revision: recreational (10%) vs. competitive (15%), P=0,337); contralateral (recreational (11%) vs. competitive (18%), P=0,236) or pivoting athletes

(revision: other (11%) vs. HSB (15%), P=0.326); contralateral (other (11%) vs. HSB (21%), P=0,173)).

There were no significant differences in patient-reported outcomes, except for the KOOS symptoms score ($P < 0.005$). Interestingly, the KOOS pain subscale score was significantly higher in the under 16-year-old athletes (P=0.033).

Our study supports the conclusion that ACL reconstruction performed during adolescence yields less favourable surgical outcomes compared to procedures carried out at an older age, particularly in terms of re-rupture rates and contralateral ACL rupture. Reducing this unfavorable outcome is of paramount importance for orthopedic surgeons, as minimizing re-rupture and contralateral ACL rupture rates is essential to improving long-term functional results in adolescent patients. One of the most promising techniques of the last decade is lateral extra-articular tenodesis (LET). This procedure has shown significant benefits in improving outcomes in ACL reconstruction, particularly for young, high-activity patients. It helps stabilize the knee, reducing re-rupture and graft failure rates among individuals with high sports activity (210-214).

Due to the characteristics of the Hungarian healthcare system, the doctor–patient relationship tends to be particularly strong in cases requiring surgical intervention. Patients also demonstrate a strong attachment to the institutions where they received their treatment and surgery. Although in many cases the follow-up contact was not made directly by the operating surgeon, the patients generally responded positively to the outreach, largely due to their trust in the institution and satisfaction with their treatment. Furthermore, patients with less favourable surgical outcomes were also motivated to participate in the follow-up examinations, as this provided an opportunity for them to receive updated information regarding the condition of their knee joint.

5.3 Possible Complications – Tunnel Widening

The most important finding of this study was the significantly lower femoral tunnel widening incidence rate following ACL reconstruction using press-fit fixed patellar tendon graft compared to using the suspensory-fixed semitendinosus graft. This is the first study to examine the effect of ACL reconstruction with press-fit fixed patellar tendon technique on femoral tunnel widening.

Except for the tibial AP view we found significantly higher tunnel widening incidence rates in the HS group on all of the radiographs (HS vs. PT: fem. AP: 89% vs. 17% $p<0.001$; HS vs. PT: fem. lat: 84% vs. 2% $p<0.001$, HS vs. PT: tib. AP: 97% vs. 94% $p=0.163$; HS vs. PT tib. lat: 97% vs. 84% $p<0.001$). The incidence rates of the tunnel widening show relatively high variety (between 0-90%)(125). Most authors agree that this phenomenon mostly occurs when using a hamstring graft. Hersekli found the rate of femoral tunnel widening to be more than twice as high using HS graft (100%) than PT graft (46%). The difference was shown at the tibial end as well, but that difference was not statistically significant (HS:PT 82%:76%) (131). In our study, the tibial widening incidence rate was also higher than the femoral incidence rate in both groups, although the femoral rates were much lower than in the referenced studies in the PT group (fem. AP: 17%, fem. lat: 2% vs. 46%, 76%).

The tunnel widening after ACL reconstruction is more often seen if hamstring graft is used – compared to patellar tendon grafts, and the widening is more pronounced in the femoral end than in the tibial (125, 130-133, 215). Except for the femoral tunnel widening on the lateral view in the PT group, all of our tunnel widening rates were significant in both groups, and the incidence rates were the highest in the HS group (HS: fem. AP:50% $p<0.001$, fem. lat: 47% $p<0.001$, tib. AP: 38% $p<0.001$, tib. lat: 41% $p<0.001$; PT: fem AP: 6% $p<0.001$, fem. lat: 0,5% $p=0.02$, tib. AP: 36% $p<0.001$, tib. lat: 28% $p<0.001$). The difference in the widening rates was observed only on the tibial AP views and was not significant between the two groups.

Several theories exist on which factors play a role in tunnel widenings, such as the different types of grafts, the movement of the graft within the tunnel, the age of the patient, accelerated rehabilitation, the size and the position of the drilled holes, the different types and devices of the graft fixation and higher cytokine activity (137-140, 142, 144, 146). Our theory is that during knee movement, the graft is stretched to the wall of the tunnel, it is compressed to the bone, and as a result, a space is created between the graft and the bone where the synovial fluid can leak into the tunnel (125). The synovial fluid contains a large number of proinflammatory cytokines (TNF-a, IL-1b, IL-6) which increase osteoclast activity, leading to bone resorption and thus, tunnel widening (149, 155, 156). We hypothesized that if the aperture of the femoral tunnel is closed with a bone block without any fixation devices and if we prevent the synovial inflow to the tunnel, it

can cause less femoral tunnel widening. This theory can be supported by the study of Hollis et al., who placed autologous bone plugs in the femoral tunnel aperture, and they found less femoral tunnel widening, although the difference was not significant (158). In case the tibial bone block was flipped next to the patellar tendon, it was closer to the joint line, decreased the synovial fluid inflow, and reduced the tibial tunnel widening significantly (155). Other authors found that the use of the graft fixation method close to the joint line can reduce the movement of the graft within the tunnel, thereby it leads to less tunnel widening (149). Fauno et al. achieved significantly less femoral and tibial tunnel widening using the transfix system with femoral and tibial PLLA interference screws compared to the endobutton suspensory fixation technique (138). Conversely, Buelow et al. reported higher femoral tunnel widening with PLA interference screw fixation than with endobutton using a hamstring graft (137). In our cases, the fixation of the femoral bone block was close to the joint line. We hypothesize that both factors lead to low tunnel widening rates and less femoral tunnel widenings.

The aim of an ACL reconstruction is to make the knee joint stable. The graft, the fixation method, and the operation technique have an important role in the success of the surgery. Using PT graft is a common technique; it has the same result as using HT or quadriceps tendon graft (216). There are a lot of possibilities for femoral graft fixation, such as interference screws, transfix method, suspensory systems or the press-fit technique. The most important questions are: does the femoral press-fit fixation method provide good graft stabilization, and can it offer good long-term result? Pavlik at al. measured satisfying failure strength using the femoral press-fit fixation at a pig ACL reconstruction (167). Arnold et al. found the same primary stability with ultimate load to failure pulls forces at least equal to published results for interference screws (217). Hertel et al. found excellent clinical results ten years after ACL reconstruction using PT graft with press-fit fixation method (218). Widuchowski et al. published the same good results at 15-year follow-up after ACL surgery using the femoral press-fit technique (219). Sarzaeem found comparable results between using the press-fit and interference screw fixation (220). Using the press-fit fixation technique yields low graft failure and revision rates (221).

5.4 Strengths and limitations

5.4.1 Genetic Analysis

A major strength of this study is the application of a GWAS approach, which allows for the unbiased investigation of genetic susceptibility to ACL rupture across the entire genome. In addition, the study included more than 600 participants, making it one of the largest genetic studies on ACL injury conducted in a Hungarian population. The rigorous phenotypic verification of ACL injuries and the strict quality-control procedures further enhanced the reliability of the findings.

This study has several limitations. Although the sample size was larger than that of most previous candidate gene studies, it remained relatively modest for a genome-wide association study, potentially limiting the statistical power to detect variants reaching genome-wide significance. In addition, no formal a priori power analysis was performed. Nevertheless, the cohort size exceeded that of many previously published genetic studies investigating ACL injury susceptibility. Furthermore, the study population consisted exclusively of individuals of Hungarian origin and European ancestry, which may restrict the generalizability of the findings to other ethnic groups. Despite adjustment for population stratification, residual confounding cannot be completely excluded. Moreover, owing to the observational case-control design, causal relationships cannot be established, and relevant environmental and biomechanical risk factors were not comprehensively assessed. Finally, the identified genetic loci were not functionally validated; therefore, their biological significance requires further investigation.

5.4.2 ACLR under 16 Years old patients

This study provides valuable information on ACL reconstruction outcomes in adolescent athletes, a population with a particularly high risk of re-injury. A notable strength is the direct comparison between patients younger and older than 16 years using standardized clinical assessments, patient-reported outcome measures, and objective stability testing. Furthermore, all patients followed the same rehabilitation protocol, reducing treatment-related variability between groups.

Preoperative patient-reported outcome measures were not available, preventing the assessment of clinical outcomes before ACL reconstruction. The follow-up period was of medium duration and varied considerably among patients, which required matching of follow-up times between groups and resulted in a reduced sample size. In

addition, later re-injuries and subsequent surgical procedures may not have been captured. A substantial proportion of patients were lost to follow-up, introducing the potential for selection bias. Furthermore, return to sport was not analyzed in detail according to sport type or level of participation. These factors should be considered when interpreting the results. Moreover, differences in follow-up duration and postoperative activity level between the groups should be considered potential confounding factors and may have influenced the observed clinical outcomes.

5.4.3 Tunnel widening

The tunnel widening analysis was strengthened by a relatively large cohort of patients and the use of standardized radiographic assessments. All surgical procedures were performed by a single experienced surgeon and all patients underwent the same postoperative rehabilitation protocol, minimizing surgeon-related and rehabilitation-related confounding factors. Additionally, the comparison of two commonly used graft types provided clinically relevant information regarding the development of tunnel widening following ACL reconstruction.

This study has several limitations. First, the study has a retrospective design, which limits causal inference Therefore, the enrollment of the patients was not randomized. There was a change in the operating technique in 2008, when the surgeon changed the type of graft from the patellar tendon to the hamstring tendon. We analyzed both groups, but by doing so, we could not ensure randomized patient enrollment. Secondly, we measured the tunnel widening on the X-ray images, rather than on CT or MRI scans. The divergence of the X-ray beams cause a magnification effect, which depends on the distance between the X-ray machine and the joint and the distance between the joint and the X-ray film cassette (149). We used standard properties for all radiographs. Therefore, the tendency is similar on the images. Nevertheless, our most important finding is the significantly less femoral tunnel widening incidence rate when using PT graft (PT: AP 17%, lat: 2% vs. HT: AP 88%, lat: 83%), and it is independent of the magnification effect. At the National Institute for Sports Medicine, an institutional change in surgical technique occurred in 2008, during which a routine transition was made to hamstring graft transplantation. In the case of an experienced surgeon, the choice of graft alone does not influence the surgical outcome; however, this technical transition may appear as a confounding factor.

5.5 Implication for practice and research

The role of genetic polymorphisms in ACL injury susceptibility has been investigated extensively; however, no definitive genetic markers have yet been identified. Should robust genetic risk factors be established in the future, genetic screening could complement anatomical and neuromuscular assessments to identify individuals at increased risk of ACL rupture. Such an approach may facilitate targeted preventive interventions, including individualized neuromuscular training and injury-prevention programs for high-risk athletes.

Future research should focus on validating the identified genetic associations in larger and more diverse populations. Furthermore, collaboration with existing genetic databases, international research consortia, and sports federations may substantially increase sample sizes and improve statistical power, thereby facilitating the identification of clinically relevant genetic risk factors for ACL injury.

Due to the high rates of re-injury and contralateral ACL ruptures observed in young, high-activity patients, it is recommended to consider surgical techniques that provide additional stability, such as LET. These procedures help improve knee stability and reduce the risk of re-ruptures and graft failures in patients engaged in high-impact sports. A saját gyakorlatunkban 2,5 éve végezzük nap rendszerességgel az LET stabilizálást fiatal korú betegeknél. Eddig 53 esetben végeztük a műtét, ahol xx átlag életkorral, és xx ál..

In recent years, the use of the quadriceps tendon has gained increasing popularity in ACL reconstruction. This graft can be harvested either as a soft-tissue graft or as a bone–quadriceps tendon graft, providing greater flexibility in surgical planning. Given the favorable biomechanical characteristics of the quadriceps tendon and the growing clinical experience with its use, future studies should investigate whether a fixation strategy combining femoral press-fit fixation with tibial interference screw fixation could achieve similarly low rates of tunnel widening. Such an approach may offer the biological advantages of aperture fixation while minimizing tunnel enlargement and facilitating potential revision procedures.

5.5

6 Conclusions

1. Which genetic polymorphisms contribute to the development of ACL rupture?

We identified suggestive association between polymorphisms in the GALNT11, ITGAM, C10orf82, OR7E47P, HSPA12A, OPRD1 and NEK10 genes and ACL tears, however, we did not find any variant with a genome-wide significant association.

2. What are the outcomes of ACL reconstruction in patients younger than 16 years of age?

Among patients under the age of 16 years, 14.1% underwent revision ACL reconstruction and 18.3% underwent contralateral ACL reconstruction, with 70% of revisions occurring within the first three years. The median time to revision was 28 months compared to 53 months for contralateral procedures, and while 31% of patients required meniscal surgery, only 43.6% had no secondary surgical interventions.

3. What is the impact of sex and athletic activity level on the surgical outcomes of ACL reconstruction?

The proportion of females was significantly greater among those who underwent contralateral ACL reconstruction. No significant differences were observed in the incidence of revision or contralateral ACL injuries between competitive and recreational athletes, nor between participants in the three major ball sports (HBS: handball, basketball, soccer) and those engaged in other sports.

4. Is there a difference in surgical outcomes between patients younger than 16 years and those older than 16 years?

The number of total surgeries, especially meniscus surgeries, was significantly greater among athletes under the age of 16 years. Contralateral and revision ACL reconstructions were also more common, but this rate was only tendentious.

5. What differences in tunnel widening are observed following ACL reconstruction using patellar versus hamstring tendon grafts, with particular attention to the incidence of femoral tunnel enlargement?

The femoral tunnel widening incidence rate during an ACL reconstruction is significantly lower when using PT tendon with femoral press-fit fixation than when using HS tendon with suspensory fixation method.

7 Summary

The ACL injury is one of the most common and serious sports-related knee injuries, causing functional impairment and long-term degenerative changes. Although ACL reconstruction is routine, graft re-rupture remains significant, especially in young, active populations. This dissertation studied selected aspects of ACL injuries and graft failure, focusing on genetic polymorphisms, outcomes in young patients and tunnel widening.

In our investigations, we performed genetic analysis among ACL-injured patients to identify potential genetic variations and created a database of patients with well-documented phenotypes. We did not find any variant with genome-wide significant association; however, we demonstrated a possible genetic link (p-values between 5×10^{-5} and 5×10^{-7}) between polymorphisms in the GALNT11, ITGAM, C10orf82, OR7E47P, HSPA12A, OPRD1, and NEK10 genes and ACL tears, suggesting that regulatory gene polymorphisms may play a more important role in ligament rupture than candidate gene variants.

In the second part of the dissertation, we analyzed outcomes of ACL reconstruction in patients under 16 years of age. We found that rates of contralateral ACL reconstruction ($p=0.03$), meniscal surgery ($p=0.003$) and total number of operations ($p=0.01$) were significantly higher compared to patients over 16 years. Although revision rates were higher in the younger group, this difference was not statistically significant. The high rate of repeat surgeries in young patients is concerning; anterolateral stabilization procedures (e.g., ALL, LET) have been shown to reduce the risk of re-rupture that is why LET stabilization has been introduced in our practice for younger patients.

The third part of the study examined tunnel widening following ACL reconstruction. Our results showed that with patellar tendon grafts and double press-fit fixation, the femoral tunnel fully incorporates in 83–97% of cases, which simplifies revision surgery and is more cost-effective due to the absence of implants.

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9 Bibliography of the candidate's publications

Related to the thesis:

1. **Tátrai M.,** Halasi T., Tállay A., Tátrai A., Pavlik A.: Low femoral tunnel widening incidence rate after ACL reconstruction using patellar tendon graft with press-fit fixation; Indian J Orthop. 2023 Feb 24;57(4):596-602. **IF: 1.1**

2. **Tátrai M.**, Halasi T., Tállay A., Tátra A., Karácsony A. F., Papp E., Pavlik A.: Higher revision and secondary surgery rates after ACL reconstruction in athletes under 16 compared to those over 16: a case-control study J Orthop Surg Res. 2025 Jun 17;20(1):597. **IF. 3.8**

Unrelated to the thesis:

1. Szijártó A, Gyurkovics E, Arányi P, Onody P, Stangl R, **Tátrai M**, Lotz G, Mihály Z, Hegedüs V, Blázovics A, Kupcsulik P. Effect of postconditioning in major vascular operations on rats].Magy Seb. 2009 Aug;62(4):180-7.

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9.

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