Genetic Insights into ACL Tears: Exploring the Association of Type 1 Collagen Polymorphisms and Anterior Cruciate Ligament Tears

By
Apurva Kannu
April 2024



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April 2024

A Senior Comprehensive Project in Partial Fulfillment of the Requirements for a Bachelor of Science Degree from Allegheny College

I hereby recognize and pledge to fulfill my responsibilities as defined in the Honor Code and to maintain the integrity of both myself and the College community as a whole.

_____

Pledge

Approval by the Senior Thesis Committee:

_____

Dr. Becky Dawson

_____

Dr. Lisa Whitenack

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ABBREVIATIONS

ACL - Anterior Cruciate Ligament

SNP - Single Nucleotide Polymorphism

COL1A2 - Type 1 Collagen gene

NCAA - National Collegiate Athletic Association

NFL - National Football League

PCR - Polymerase chain reaction

Mvmnt - Movement (of injury)

Piv. - Pivoting

Rot. - Rotation

ABSTRACT

ACL tears are becoming a more severe and prevalent problem among athletes, with negative impacts on physical, mental, and emotional health. The injury removes the stabilization between the femur and tibia and the rotational stability of knee function. Many factors impact the mechanics and risks of ACL ruptures. However, not much is understood about genetic predispositions and their role in injuries, as some athletes are more vulnerable to tears despite similar playing conditions. This study investigated the potential association between single nucleotide polymorphisms in a Type 1 collagen gene, *COL1A2,* and the increased risk of ACL tears. Due to limitations in the TaqMan assay, the PCR amplification results could not be directly applied. Despite the challenge, samples with amplification suggested a need for further exploration into genetic insight in ACL ruptures. The study emphasized the need for future studies on SNP genotyping methods to enhance our understanding of genetic predispositions influencing ACL injury risk.

INTRODUCTION

Anterior cruciate ligament, ACL, tears are among some of the most severe and debilitating sports injuries. The anterior cruciate ligament is a collagen-heavy ligament located in the center of the knee between the femur and tibia. It is responsible for the function and stability of the knee to prevent it from shifting or dislocating during movement. It is a strong band of connective tissues that cross to form an "X" shape inside the knee (Evans et al., 2023). Starting from the corner of the femur (thigh bone), the ligament inserts into the tibia (shin bone) to provide the connection. Mechanics and functions of the ACL include knee flexion, stabilization against rotation, protection from forces of blow to the knee, and mechanoreceptors responsible for coordination. Despite preventative care, ACL tears and re-tears are increasing at an alarming rate of 26% as of 2022 (Minjares., 2023). Several factors contribute to the seriousness and nature of the injury: pain and disability, risk of further damage, prolonged recovery time, financial costs, and impact on mental health (Haugen., 2022). Understanding possible risk factors associated with ACL tears is crucial in taking steps toward identifying higher risk factors and improving injury prevention.

One possible association of ACL injuries is genetic predispositions. The ligament is primarily comprised of collagen, with 85% being type 1 collagen. *COL1A2* is a type 1 collagen gene discussed in the literature as having single nucleotide polymorphisms that can affect the expression and tissue function of the ligament (Perini et al., 2022). Further analysis of *COL1A2* shows evidence of an association between single nucleotide polymorphisms (SNPs; mutations caused by single base substitutions) and non-contact ACL tears due to possible tissue failure in the changing of gene expression of collagen (Kaynak et al., 2017). While a 2022 study in Brazil

focused on two collagen genes, the study done here focused on *COL1A2*, which is likely to change the protein structure of collagen (Perini et al., 2022).

Based on the composition of the ACL and what is known about polymorphisms in *COL1A2*, I hypothesize that athletes who have torn their ACLs will present with the single nucleotide polymorphism in *COL1A2*, while those who have not suffered a musculoskeletal injury will not. The hypothesis will be tested through a split study design. Using a questionnaire, health history, injury information, and athletics information will be collected as variables associated with the presence of a possible polymorphism. The experimental approach involves DNA extraction, SNP genotyping, PCR amplification, and descriptive analysis to determine a potential association.

BACKGROUND & SIGNIFICANCE

Understanding the possible factors associated with ACL tears is crucial for injury prevention and improvement.

Unfortunately, ACL tears are rising among athletes who specialize in their sport. They lead to physical stress, psychological impacts, financial costs, and time loss from athletic activity. It is the most common injury in the NCAA (National Collegiate Athletic Association), requiring surgery, and has a low rate of return to pre-injury performance level (Ptasinski et al., 2022). Athletes at every level of competition are experiencing this painful and time-loss injury. The average number of ACL tears per season in the NFL (National Football League) has been 41.7 since 2010 (Linder., 2023). ACL tears are still common even at the most competitive level of football, where there is access to top medical treatment and injury prevention. Within the NCAA, there are discrepancies between resources available. At the D3 level, athletes face limited access to sport-specific training programs, injury prevention techniques, limited sports medicine staff, and lower quality of rehabilitation following injuries than in the higher two divisions (Anderson et al., 1994).

Additionally, athletes at this level are understudied for sports as they face a holistic lifestyle emphasizing academics, employment, and extracurriculars. Reporting ACL injuries is substantially more difficult at the D3 level because it is the largest NCAA division with 430 schools and over 200,000 student-athletes (NCSA Sports). With limited resources and an understudied population, research into factors causing ACL tears is needed for D3 athletes.

With a rate of return to sports just under 50%, it is becoming increasingly important to strengthen preventative measures, be preemptive, and identify factors that put athletes at greater risk of ACL ruptures (Joreitz et al., 2020). Multiple physical, emotional, and mental factors

contribute to such a low rate of return. The most significant impact is time loss and psychological factors. Over sixty percent of athletes do not return to their sport 12 months after surgery (Christino et al., 2016). The time loss itself is associated with depression, anxiety, low self-esteem, and pain (Christino et al., 2016). Fears concerning post-op performance, lower knee function, and re-injury prevent many athletes from staying consistent with rehabilitation and physical therapy. Even then, limited resources at the D3 level can make routine follow-ups, sport-specific rehabilitation, and access to physical therapy equipment difficult. Designing injury prevention and treatment programs for each sport based on the movements is another limitation many D3 schools do not have the luxury of doing.

While many factors are identified to impact ACL injury risk, the only definitive information understood is when and where the injury can happen. However, it is still not clear why many athletes who play under the same conditions (sport, training, age, and sex) still suffer ACL tears. The variability of injury, even within a homogenous group of athletes playing under the same circumstances, has led to questions about the association between the risk of ACL tears and genetic predispositions. However, studies and literature on genome association with injury need more substantial research and studies (Slodkowska et al., 2015).

COL1A2, a type 1 collagen gene, plays a crucial role in producing collagen, a molecule that strengthens and supports tissues, ligaments, and cartilage in the body. Given that the ACL is 85% collagen, there is a potential link between this gene and the susceptibility to ligament tearing (Fukusato et al., 2021). Further analysis of *COL1A2* reveals evidence of an association between single nucleotide polymorphisms (SNPs; mutations caused by single base substitutions) and non-contact ACL tears. SNPs can be further described as a variation in a single base pair from a substitution, deletion, or insertion in a DNA sequence, resulting in a mutation. These

SNPs can lead to tissue failure and alter the gene expression of collagen (Perini et al., 2022). Within SNPs, there are variations in the genome that can affect the phenotypic impact between healthy individuals, while those who are predisposed to duplication or deletions can present with the disease (Barøy et al., 2008). Identifying specific substitutions in *COL1A2* is crucial as certain alleles in the gene can translate to other alleles through RNA transcription, affecting the strength and stability of specific proteins produced. If the proteins in the cell are altered, then the overall stability of the collagen can be affected. Changes and deficiencies in collagen can impact the biomechanics of knee movement and increase the risk of ACL tears (Zdzienlik et al., 2021). Athletes aware of this mutation can reduce the risk of injury through a more accurate and targeted injury prevention routine and consult with trainers and health care experts on injury prevention methods. This knowledge empowers athletes to take control of their health and well-being, potentially reducing the incidence of ACL tears in non-contact sports.

The literature notes that the relationship between ACL injuries and gene polymorphisms still needs to be fully understood. However, it provides insight into the importance of genetics, their role in injuries, and how that can be used to advance prevention approaches (Astur et al., 2017). This study aims to fill this gap, providing athletes with a better understanding of their genetic predisposition to ACL tears and empowering them to take proactive steps in injury prevention.

I hypothesized that the single nucleotide polymorphism in *COL1A2* would be more prevalent in athletes who have torn their ACLs. The results add more knowledge to a new and lacking area of research. Additionally, using information such as polymorphisms in athletes can help understand one of many factors associated with ACL tears. This study can open future research and improvements to injury prevention, treatments, and sport-specific training.

METHODS

(1) ACL tear and injury history questionnaire

This study was conducted through two methods: 1) Questionnaire survey collecting responses from athletes that explored multiple factors in an injury and 2) Experimental study using DNA extraction, SNP genotyping, PCR amplification, and descriptive analysis to confirm the presence or absence of the polymorphism in the athlete's gene. Both the questionnaire and spit study were approved by the Allegheny Institutional Review Board. The survey gathered insight into possible variables affecting an ACL injury that might be associated with the polymorphism. Sex, injury history, injury leg (right or left), movement of injury, nature of injury (contact or non-contact), and other ligament injuries were collected as factors that could have links to the polymorphisms in increasing the risk of ACL tears. As a disclaimer, sex refers to biology and not gender identity.

Injury history was collected to understand better possible factors that put the athlete at a higher risk of tearing their ACL if they had other previous ligament injuries indicating their injury proneness. Movement of injury (pivoting, jumping, hyperextension, and deceleration) provides further insight into how a genetic predisposition could affect the collagen and which specific movements are associated with the possible polymorphism. Establishing if the injury was contact (a direct blow to the knee causing it to bend inward) or non-contact (pivoting, landing, jumping, and twisting) is essential in determining which athletes are at a higher risk of injury based on their sport (contact or non-contact).

For Allegheny College students, the survey included the option to participate further by providing a spit sample. If participants selected yes, a second link was provided after submitting

the questionnaire to collect emails for samples. This study consisted of 21 survey responses and 5 participants for the experiment.

(2) Sample collection

A Salivette tube, a sterile cotton roll inside an exterior tube, was used for saliva collection (Figure 1). The double tube organization of the saliva tube allowed the participants to obtain their own saliva samples, resulting in the most saliva volume. Using gloves, participants placed the cotton roll in their mouths for two minutes and then returned it to the interior tube. The cap placed on the tube will wring the saliva into the bottom of the exterior tube. Due to the amount of saliva produced, the five samples were replicated. The ten samples were placed in a -20° Celsius freezer.



Figure 1. Sarstedt Salivette collection tube assembled (left). Disassembled into exterior tube, inner tube, cotton roll, and cap. (right). (FisherScientific.com, 2024).

(3) DNA Extraction

A Genomic DNA Purification Column was used to extract the DNA from the saliva. Using Thermo Fisher's manual for recommended volumes based on the sample tissue, 20 microliters (µL) of RNase A solution was added to 500 µL of each spit sample. The samples

were then vortexed and incubated at room temperature for ten minutes. 200 µL of Lysis solution was added and vortexed for fifteen seconds. 400 µL of 50% ethanol was added and mixed by vortexing in prep for transfer to the purification column enclosed in a collection tube. After transferring the lysate to the purification column, the samples were centrifuged for 1 min at 6000 RCF (g force). The flow-through solution at the bottom of the collection tube was discarded, and the column was placed into a new, sterile 2 mL collection tube. The following steps involve washing the samples with buffer solution. 500 µL of Wash Buffer 1 was added to the samples in the column, centrifuged, and the flow-through solution was discarded while keeping the collection tube. 500 µL of Wash Buffer II was added to the purification columns and centrifuged for 3 minutes at 12,000 g. The collection tube containing the flow-through solution was discarded, and the purification column was added to a sterile 1.5 mL microcentrifuge tube. 200 µL of Elution Buffer was added to the center of the purification column to elute genomic DNA (SOURCE). The samples were incubated for 2 minutes at room temperature and centrifuged for 1 minute at 8,000 g. Finally, the purification column was discarded, and the extracted DNA was at the bottom of the 1.5 mL microcentrifuge tube. Samples were immediately stored at -20° Celsius in preparation for SNP genotyping the following day.

(4) SNP Genotyping and PCR amplification

The TaqMan SNP genotyping assay and PCR amplification are commonly used in studies with SNP genotyping of tissues (Bell et al., 2012). The TaqMan assay protocol uses probes to identify and target the polymorphism on the genomic sequence (Thermofisher, 2017). Using Thermo Fisher's manual, the following volumes were suggested for the reaction: 5 µL of Master Mix (mix containing target-specific primers and probes), 2.5 µL of purified DNA, 0.5 µL

TaqMan assay, and 2 µL of nuclease-free water. First, the reaction mix was prepared with 5 µL of Master Mix added to 0.5 µL TaqMan assay solution. The reaction mix was vortexed and centrifuged to ensure proper mixing, and the 5.50 µL mix was pipetted into each tube of a PCR strip. Two PCR strips were used for the ten samples, as one strip contains eight wells. The strips were placed onto a PCR tray for centrifuging to bring the contents down. 2.5 µL of the purified DNA was added to each well containing the master mix. After centrifuging one final time, the ten reactions were prepped for PCR amplification. The Bio-Rad CFX Opus Real-Time PCR System was utilized for the amplification and included a feature to save a program file with the set cycling conditions. The TaqMan assay manual provided the cycle conditions for each step. The first denaturation was at 95° C for 10 minutes, followed by 40 cycles of denaturation at 92° C for 15 seconds, and the last cycle of annealing at 60°C for 1 minute (Thermo Fisher., 2014). The TaqMan assay is labeled with VIC and FAM reporter dyes, each associated with one specific allele (Res., 2001). The total PCR amplification reaction time was 1 hour and 27 minutes. Once completed, a USB flash drive was inserted to download and save the amplification results.

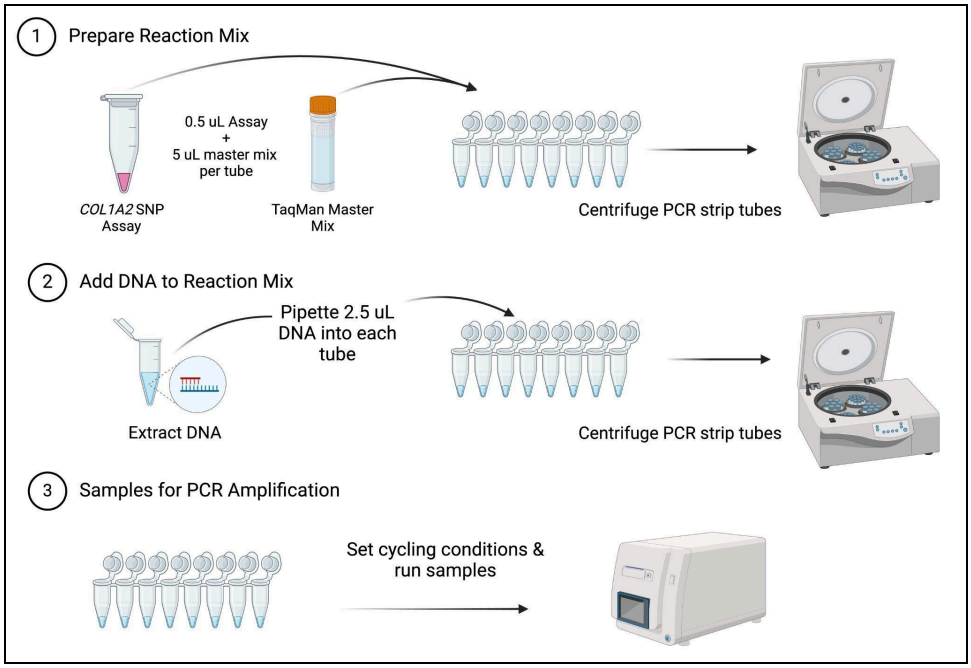


Figure 2. Methods of SNP genotyping using TaqMan assay prior to PCR amplification. *Created with BioRender.com (2024).*

(5) Descriptive Analysis

Results from the survey sample were aggregated and displayed in Table 1. Variables known to be associated with ACL injuries were selected for the descriptive analysis. The samples were split into two groups: tears (Y) and no tears (N). The survey data was divided into the following variables: sex (M/F), Leg (R/L), movement of injury (pivot, jump, hyperextension, deceleration, or other), contact or non-contact injury, and other ligament injuries. The final variable group was the presence or absence of the polymorphism (Y/N). The results of the PCR analysis were conducted. Cq values, the number of cycles until DNA is amplified, were obtained for the analysis. The maximum number of cycles in PCR amplification is 40 cycles.

RESULTS

(1) Injury-related questionnaire

Five cases were included in the sample population—three samples presented with ACL tears due to a non-contact sports injury (60%). Two tears resulted from a pivoting movement (66%) and one from rotation (33.3%). Additional factors regarding ACL ruptures are displayed in Table 1. Variables in the survey would have been used in statistical analysis as possible confounding variables of an association between ACL tears and *COL1A2*.

	Sex (M/F)	ACL Tear	Leg (L/F/Both)	Mvmnt	Sport/ Non-sport	Contact/ Non-Contact	Reinjury	Other Lig.
A1	F	Y	L	Rot.	Sport	Non-cont.	N	Y
A2	F	Y	Both	Piv.	Sport	Non-cont.	N	Y
A3	F	Y	L	Piv.	Sport	Non-cont.	N	Y
A4	F	N						
A5	F	N	L					Y

Table 1. Selected confounding variables known to be associated with ACL ruptures collected from the injury questionnaires of five participants in the spit sample population: sex (M = male/F = female), ACL Tear (Yes/No), Leg (Left, Right, or both), Mvmnt (movement on injury: pivoting, rotation, hyperextension, and jumping), Contact injury or non-contact, any occurrence of reinjury, and other ligament injuries (meniscus, PCL, and MCL tears).

(2) PCR Cycles: Cq Values

Following PCR amplification, the haplotype of every sample indicated a heterozygote call. The call indicates that two alleles on the gene locus were amplified. Cq values indicate how many cycles were completed for amplification to result. Only the results of the FAM (Fluorescein) fluorophore were analyzed. Cq values ranged from 32.62 to 37.37 cycles (Table 2). The mean Cq value was 35.198 cycles. Only two samples, *A3 and A4,* had Cq values under 35.00

cycles and were considered amplified. The remaining eight samples had Cq values above 35 cycles (with the exception of sample B1, with a Cq value of 34.59). They were unusable due to low DNA concentration or background noise during amplification. Samples A3 and A4 had a presence of DNA to be amplified, yet not enough to conclude results that indicated a polymorphism or not.

Sample	Fluorophore	Cq	Sequence	Tear
A1	FAM	35.07	N/A	Y
A2	FAM	37.37	N/A	Y
A3	FAM	33.85	Amplified	Y
A4	FAM	32.62	Amplified	N
A5	FAM	36.21	N/A	N
B1	FAM	34.59	N/A	Y
B2	FAM	35.11	N/A	Y
B3	FAM	36.41	N/A	Y
B4	FAM	35.51	N/A	N
B5	FAM	35.24	N/A	N

Table 2. Cq values were obtained from ten reactions following PCR amplification. Fluorophore indicates the fluorescent dye FAM, Fluorescein amidites, targeting *COL1A2*. Sequence amplification results are shown only in samples A3 and A4. ACL tear (Y/N) was indicated per reaction collected from the injury survey.

DISCUSSION

ACL tears are a severe time-loss injury with negative physical, emotional, and mental impacts on athletes. The etiology of these ruptures continues to go understudied and misunderstood today, especially among D3 college athletes. This study aimed to highlight lacking research into the role of genetic predispositions on the risk of ACL injuries. The findings of this study highlight a few gaps in research and methodologies exploring the association. In Table 2, it was observed that only two out of the ten samples had a slight amplification of the SNP in *COL1A2*. Despite the small amount of amplification, it continues to indicate the presence of the targeted SNP in *COL1A2* and emphasizes the importance of exploring genetic impacts on injuries. The primary goal was to identify the SNP in *COL1A2*. However, the predesigned TaqMan assay targeted a different SNP from the literature. The SNP identified in the literature (Perini et al., 2022) did not have a predesigned assay from Thermo Fisher. Perini et al. would have had to design their own TaqMan assay in-house with the genomic sequence of *COL1A2* and the specific locations of the SNPs in the sequence. Due to this difference, results on SNP amplifications could not be applied using the same protocol. Despite these differences, the findings from this study are significant as they suggest refining methods of SNP genotyping and other challenges faced during DNA collection.

For future recommendations, the Sarstedt salivette collection system for collecting saliva should not be utilized. While the method allows participants to self-collect, the cotton roll prevents proper drainage and collection of enough DNA. Additionally, the salivette system required a second trial due to the lack of saliva in the initial collection. Future studies should explore using a passive drool method to allow for the highest concentration of DNA. Considering this study's findings, future studies should further explore which specific SNP of

COL1A2 is being targeted in the TaqMan assay. It should be acknowledged that studies into specific SNPs of *COL1A2* affecting collagen production, which can be associated with ACL tears, are limited to one case study in Brazil.

Research into SNPs of *COL1A2* associated with collagen gene expression is also lacking. This emphasizes the importance of confirming which SNP is targeted during SNP genotyping. To ensure the protocol performed in literature can be duplicated for further research into genetic predispositions, the exact SNP location on the genome sequence of *COL1A2* must be identified and sent to Thermo Fisher to obtain a designed TaqMan assay kit for genotyping. Ensuring this would allow amplification results to be utilized in statistical analysis to investigate possible associations between single nucleotide polymorphisms and ACL ruptures.

(1) SNP Identification

The SNP targeted in this study (rs148802548) has no existing studies or reports in any literature (National Institutes of Health, 2022). In one case-control study in Brazil, the SNPs identified from *COL1A2* (rs42524) associated with an increase in ACL tears differ from the SNP identified in this study. The difference between these SNPs is what they can exhibit on *COL1A2* based on their specific locations and variants. For this study's results to be interpreted similarly to the Brazilian study's protocol in literature, the TaqMan assay needed to be designed for the SNPs mentioned in the literature. However, Thermo Fisher did not have a predesigned assay kit for this SNP, which researchers in Rio De Janeiro most likely designed in-house for their study. For this study, sending the genetic sequence to Thermo Fisher and requesting a specific kit to be designed would have results interpretable based on protocols from the literature. However, the customization of identifying the primers and probes for the particular location in the DNA

sequence was out of the scope of this study. As a result, no definitive conclusions can be made regarding the samples in this study in terms of what was observed in the literature. For future studies exploring genetic predisposition with ACL tears, the genomic DNA sequence of the SNP being studied should be sent to Thermo Fisher to request an assay to be designed. In addition, a control sample of DNA with polymorphism should be used to assess the presence or absence of polymorphism.

(2) Cq Values

From the ten reactions of PCR amplification, only two samples had Cq values under 35 cycles used for descriptive analysis. Cq values indicate the number of cycles it took for the DNA to be amplified, with the max number of cycles set at 40. The higher the Cq value, the less amplification there is (Ruiz-Villalba et al., 2021). The eight samples resulting in over 35 cycles could not be used and can be attributed to a low DNA concentration or background noise during PCR amplification. The two samples under 35 cycles were considered to be amplified. A lower Cq value indicates a higher amount of target DNA in the sample and suggests that the target sequence (polymorphism) could be present (Karlen et al., 2007). However, since the values are still above 30 cycles, there is more likely to be a low DNA concentration to determine a polymorphism accurately. Noting that the SNPs in the literature were distinct from the SNP in this study, a threshold Cq value could not be determined based on the protocol in the single case study. Without a threshold Cq value, it is difficult to determine which samples are being amplified over a set Cq threshold. While determining the presence or absence of the polymorphism could not happen due to no Cq threshold level, the little amplification seen at this specific SNP (rs148802548) continues to indicate that genetic predispositions could still play a factor in the etiology of ACL ruptures. The variance in the dye frequencies of FAM (RFU

values) suggests a difference in the allelic frequencies between samples. The inability to follow the standard protocol highlights gaps in current knowledge and techniques that prevent a comprehensive understanding of genetic impacts on ACL injuries.

(3) Other limitations

It is essential to consider other limitations and challenges observed in this study, given that the data regarding the SNP targeted by this TaqMan assay could not be applied. The first primary concern was the sample size. While the study required participants with ACL tears, it also needed controls of those who had never had an ACL injury. After having the survey available for 11 weeks, the questionnaire collected data from twenty-two students. Nine students from that population chose to participate further and provide a spit sample. However, four participants changed their minds due to personal reasons. The survey was kept open for one more week to continue recruiting, yet could not get any more participants. The five saliva samples were duplicated to yield more PCR amplification reactions. For future directions, advertising the survey to all Allegheny students, not solely student-athletes, should be emphasized. Since a control group of non-ACL tears is needed, this would ensure the study is not limited to specific groups on campus. Additionally, staying persistent in emailing athletic coaches and biology professors must be prioritized. With current online and on-campus resources, future researchers should take advantage of social media to reach larger audiences.

The low DNA concentration in the samples can be attributed to the Salivette collection system. While the double-tube cotton roll system was highly recommended, there was an issue with the volume of DNA collected at the bottom of the tube. Participants were instructed to saturate the cotton roll in their mouths for two minutes. When placed back in the inner tube, the cap on top compresses the cotton roll to drip the saliva to the bottom of the exterior tube. During

the initial collection, participants followed the manufacturer's instructions not to chew the cotton roll. However, no visible drop of saliva was collected after placing the cap stopper on top. Participants were told to thoroughly chew and saturate the cotton in the second trial for two minutes. This resulted in about 0.5 mL of saliva. While the second collection was significantly better, it is essential to acknowledge that the lack of consistency and allowing each participant to handle their collection could have impacted the amplification results of the samples. Alternative methods for saliva collection should be considered in future studies. The passive drool method is the gold standard that allows participants to salivate through a straw and into a vial, preventing the use of cotton rolls that could alter the sample (Fernandes et al., 2013). This method would also allow for a more concentrated DNA sample without contamination from the cotton swab.

(4) Conclusion

This study of SNP genotyping of *COL1A2* exploring SNP rs148802548 encountered challenges due to using a TaqMan assay predesigned for a different SNP (rs42524) as per the case study presented in the literature. This distinction prevented a Cq value threshold from being established post-amplification per the case study protocol. Despite this limitation, few samples displayed amplification, highlighting the necessity for further investigation into the role of genetic predispositions in anterior cruciate ligament (ACL) ruptures. The genotyping method conducted in this study underscored the need for additional research into redefining methods for SNP genotyping. Therefore, it is critical to delve deeper into exploring genetic insights associated with ACL tears and to strengthen the efficiency and accuracy of SNP genotyping protocols. The findings of this study provide insight into a significant research gap concerning methods and protocols investigating genetic factors and their impact on ACL tears. While there is a current understanding of the multifactorial impacts of ACL injuries, including environmental

and biological, there remains an insufficiency in studies exploring specific genetic predispositions associated with ACL ruptures. Understanding how variations in a gene like *COL1A2* can impact collagen's integrity regarding tensile strength and vulnerability is essential for improving injury prevention, surgical treatments, and approaches in athletics and medicine.

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APPENDIX

Appendix I: Survey questionnaire collecting injury information and history, and continued survey for email collection regarding spit sample.

ACL Injury Survey for Student Athletes

Thank you for participating in this survey. The purpose of this survey is to gather anonymous information about your ACL injury and experiences with it to help better understand the risk factors associated with the specific injury. Your participation is completely voluntary, and completion of the survey will automatically enter you in a raffle to win a $25 Amazon gift card. *This survey has been approved by the Allegheny College IRB*

Section 2: Personal Information

Personal Information: Please note that all information is anonymous and de-identified. Disclaimer: The term sex is used about the biological nature of the participant and does not adhere to gender identity.

Sex:

- ☐ Male
- ☐ Female

Grade/Year in College

- ☐ First Year
- ☐ Second Year
- ☐ Third Year
- ☐ Fourth Year

Section 3: Sport(s) & Injury History

Have you ever experienced an ACL injury?

- ☐ Yes
- ☐ No

Which leg did the injury occur on (Right or Left?)

- ☐ Right
- ☐ Left

What year did the ACL injury/injuries occur?
Short answer text

How did the ACL injury happen? (Movement)

- ☐ Pivoting/Cutting
- ☐ Jumping
- ☐ Hyperextension

- ☐ Deceleration
- ☐ Other…

What were you doing when the ACL injury occurred?

- ☐ Sport
- ☐ Other…

Was the movement/sports contact or non-contact?

- ☐ Contact
- ☐ Non-contact

Did you sustain any re-injury?

- ☐ Yes
- ☐ No

Have you sustained any other ligament or tendon injury?

- ☐ Yes
- ☐ No

If the answer to the above question is Yes, please elaborate on the injury
Short answer text

Are you a student at Allegheny College?*

- ☐ Yes
- ☐ No

For Allegheny College students only
Description (optional)

Would you be willing to participate further in this study by providing a spit sample?

- ☐ Yes
- ☐ No

Continued from ACL Injury Survey
Thank you for completing the survey and for participating further in this study by providing a sample. Please enter your email below to be entered on the list of participants. You will receive an email soon with information on the sampling process and schedule a time to fill out the informed consent & provide the sample. *This survey has been approved by the Allegheny College IRB*

Please enter your email below:*

Short answer text

This work is mine unless otherwise cited: Apurva Kannu

Allegheny College, 2024