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The Association of Collagen 1A1, 5A1 and 12A1 Gene Expression with General Joint Laxity in Athletes is Non-Significant

Sporcularda Kollajen 1A1, 5A1 ve 12A1 Gen Ekspresyonlarının Genel Eklem Laksitesi İle İlişkisi Anlamlı Değildir

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ABSTRACT

Objective: Generalized joint laxity is a connective tissue disorder, and may cause musculoskeletal injury in athletes. The gene expression levels of type I, V, and XII collagens affect the components and properties of connective tissue. Therefore, in this study, we aimed to investigate the COL1A1, COL5A1, and COL12A1 gene expression levels, which have an effect on connective tissue properties and were previously associated with ligament injuries in athletes and assess their association with generalized joint laxity.

Materials and Methods: 20 athletes were included in this study. Joint laxity was evaluated according to the Beighton Horan Joint Mobility Index (BHJMI). The participants were divided into two groups as non-hypermobile (n=11) and increased mobility and hypermobility (n=9) according to their BHJMI scores. The real-time polymerase chain reaction was used to determine the COL1A1, COL5A1, and COL12A1 gene expression levels.

Results: There were no significant differences in the relative gene expressions of COL1A1, COL5A1, or COL12A1 between the groups.

Conclusion: The gene expression levels of collagen types I, III, and V of participants with and without generalized joint laxity were not different. Genome-wide studies are recommended to evaluate the potential genetic variants associated with hypermobility, which causes sport-related injuries.

Keywords: hypermobility, collagen, gene expression

ÖZ

Amaç: Genel eklem laksitesi bir bağ dokusu bozukluğu olup, sporcularda kas-iskelet sistemi yaralanmalarına neden olabilir. Tip I, V ve XII kollajenlerin gen ekspresyon seviyeleri bağ dokusunun bileşenlerini ve özelliklerini etkilemektedir. Bu nedenle, bu çalışmada bağ dokusu özellikleri üzerine etkisi olan ve daha önce sporculardaki ligament yaralanmaları ile ilişkisi gösterilmiş olan COL1A1, COL5A1 ve COL12A1 gen ekspresyonlarının düzeylerinin ve bu gen ekspresyonlarının genel eklem laksitesi ile ilişkisinin araştırılması amaçlanmıştır.

Gereç ve Yöntemler: Toplam 20 sporcu çalışmaya dahil edildi. Eklem laksitesi Beighton Horan Eklem Mobilite İndeksine (BHJMI) göre değerlendirildi. Katılımcılar BHJMI skorlarına göre hipermobilitesi olmayan (n=11) ile artmış mobilite ve hipermobilitesi olan (n=9) olmak üzere iki gruba ayrıldı. COL1A1, COL5A1 ve COL12A1

gen ekspresyon seviyelerini saptamak için gerçek zamanlı polimeraz zincir reaksiyonu yöntemi kullanıldı.

Bulgular: Gruplar arasında COL1A1, COL5A1 veya COL12A1'in gen ekspresyonlarında anlamlı fark bulunmadı.

Sonuç: Genel eklem laksitesi olan ve olmayan katılımcıların kollajen tip I, III ve V gen ekspresyonları arasında anlamlı fark bulunmadı. Spor yaralanmalarına sebep olan hipermobilite ile ilgili potansiyel genetik varyantların değerlendirilmesi için genom boyutunda ilişkilendirme çalışmaları önerilmektedir.

Anahtar Sözcükler: hipermobilite, kollajen, gen ekspresyonu

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INTRODUCTION

The relationship between polymorphisms and susceptibility to tendon and ligament injuries is a subject that has been explored in recent years (1). Type I collagen, the most abundant protein in the body, is synthesized by fibroblasts, osteoclasts and odontoblast (2). It consists of collagen 1a1 and collagen 1a2 polypeptides in a ratio of 2:1, which are encoded by the COL1A1 and COL1A2 genes, respectively (3). COL1A1 polymorphism reduced the risk of anterior cruciate ligament (ACL) rupture and shoulder dislocation (4,5).

Type V collagen takes part in the structure of tendons and ligaments, and the α1-chain of this collagen is encoded by the COL5A1 gene (6). Studies have shown the relation between COL5A1 polymorphism and ACL rupture, Achilles and quadriceps tendon injuries (7–10). Type XII collagen is a member of the FACIT (fibril-associated collagens with interrupted triple helices) collagen family (11).

Type XII collagen is encoded by the COL12A1 gene and regulates the fibril thickness (12). In a study, the COL12A1 specific genotype was overrepresented in women who had ACL rupture (13).

Generalized joint laxity (GJL), or hypermobility, is a connective tissue disorder in which the mobility of joint increases further than normal range (14). GJL is hereditary, and familial predisposition to GJL has been shown in several studies (15–18). Higher GJL incidence in monozygotic twins compared to dizygotic twins also supported the effect of heredity (19). In a study, Bell et al. aimed to determine the association between single-nucleotide polymorphisms within the COL1A1, COL5A1, and COL12A1 genes previously associated with ACL rupture and the GJL (20). The authors found that the aforementioned gene variants previously associated with ACL injury risk were also associated with joint laxity (20). Athletes with GJL have a higher risk of injury and states of anxiety, as well (21).

The polymorphisms in the COL1A1, COL5A1, and COL12A1 genes are associated with ACL rupture, shoulder dislocation, Achilles, and quadriceps tendon injuries (4,5,7–10,13). Besides, the gene variants of type I, V and XII collagens may affect the components and properties of connective tissue (20). Previous studies, which evaluated the association between the aforementioned genes and GJL, have shown that single nucleotide polymorphism within these genes may manifest as GJL. However, it is controversial whether there is also an association between the expression levels of these genes and GJL. Therefore, we aimed to investigate the expression levels of COL1A1, COL5A1 and COL12A1 genes, which have an effect on connective tissue properties and were previously asserted to be associated with ligament injuries in athletes and assess their association with GJL.

MATERIAL and METHODS:

Design of the Study

Joint laxity of athletes, who applied to the sports medicine outpatient clinic, were assessed between June and December 2017, prospectively. All athletes were American football players, above 18 years old and training above 3 hours

per week. Athletes with history of musculoskeletal injury and connective tissue disorders were excluded. Female contributors had regular menstrual cycles and no hormone-based medication history. The study was approved by the local ethics committee of the Hacettepe University Ethics Committee (Decision number: GO 16/303). All participants were informed about the procedure, and they provided written consent, which was in accordance with the Helsinki Declaration.

Joint Laxity Assessment

Joint laxity assessment has been performed according to the Beighton Horan Joint Mobility Index (BHJMI) (22,23) that included five maneuvers: (1) Thumb opposition test: Passive opposition of the thumb to the flexor aspect of the forearm; (2) Fifth finger hyperextension test: Passive dorsiflexion and hyperextension of the fifth MCP joint beyond 90°; (3) Elbow hyperextension test: Passive hyperextension of the elbow beyond 10°; (4) Knee hyperextension test: Passive hyperextension of the knee beyond 10°; (5) Palms on the floor test: Active forward flexion of the trunk with knees fully extended so that palms of the hands rest flat on the floor. All the maneuvers above, except the palms on the floor test, were done for the right and left sides of each patient, and if the conditions were met, a score of 1 was given. The lowest score that can be taken according to BHJMI is 0, and the highest score is 9. According to their BHJMI scores, the participants were divided into two groups as non-hypermobile (NHM) (0-4), and increased mobility and hypermobility (IHM) (5-9).

Blood Collection

A 4.5 ml sample of venous blood was obtained into a vacutainer tube that contains ethylenediaminetetraacetic acid (EDTA) during the morning hours to assess collagen gene expression levels of athletes. Blood samples were quickly transferred to the laboratory.

Ribonucleic Acid Isolation

Ribonucleic acid (RNA) was extracted from blood samples with High Pure RNA Isolation Kit (Roche Diagnostic, Switzerland) and red blood cell lysis buffer (Roche Diagnostic, Switzerland) according to the manufacturer's instructions. In order to obtain the maximum RNA load from the blood samples, the RNA isolation procedure was performed on an ice block immediately after blood collection. RNA isolation was initiated within the first 30-60 min. RNA concentration and purity were assessed using NanoDrop equipment (NanoDrop 2000c Thermo Fisher Scientific, USA). The range of 6.70 to 22.72 µg RNA copies per ml blood was considered sufficient for the study. Pure RNA samples were stored at -80^{0}C until the PCR procedure was performed.

Complementary Deoxyribonucleic Acid Synthesis

The total RNA was converted to the complementary deoxyribonucleic acid (cDNA) in a thermal cycler (Bioneer MyGenie 96 Thermal Block, South Korea) with the Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostic, Switzerland) according to the manufacturer's instructions.

Real-Time Polymerase Chain Reaction

All real-time polymerase chain reaction (PCR) assays were performed using the Light Cycler 2.0 (Roche Diagnostic, Switzerland) instrument. The Light Cycler Catalog Assays (Roche) designed for COL1A1, COL5A1, and COL12A1 were used to prepare PCR mixtures. Each catalog assay included gene specific primers and a universal probe library (UPL) probe, which is a short FAM-labeled hydrolysis probe containing locked nucleic acid. This was a ready-to-use single PCR assay, enabling the quantification of gene expression levels using real-time PCR (Roche catalog assay: 05532957001). Gene expressions were investigated by real-time PCR (Light Cycler 2.0, Roche, Switzerland). The ß-Actin gene was also amplified with the same method as a housekeeping gene. Final PCR mix was prepared as follows: 10 µl master mix; 4 µl PCR water; 1 µl catalog assay; and 5 µl cDNA. The samples were submitted to the following PCR conditions: 95^{0}C for 10 min. for pre-denaturation; 45 cycles at 95^{0}C for 10 s for denaturation; 60^{0}C for 30 s for annealing; 72^{0}C for 1 s for extension; and 40^{0}C for 30 s as a final step.

Statistical Analysis

Statistical analyses were performed using the SPSS software version 21 (SPSS, Chicago, IL, United States). The variables were investigated using visual (histograms, probability plots) and analytical (Kolmogorov-Smirnov test) methods to determine normal or non-normal distribution. Descriptive analyses were presented using medians, minimums, maximums and frequencies. The Mann-Whitney U test was used to compare non-normally distributed variables. The Chi-square test was used to compare proportions in different groups. A 5% type-I error level was used to infer statistical significance.

RESULTS:

There were three females, eight males in the NHM group and four females, five males in the IHM group, with no statistically significant difference between them (p=0.4) (Table 1).

Table 1. Characteristics of participants

Parameters	NHM (n= 11)	IHM (n= 9)	P
Female, n (%)	3 (27.3)	4 (44.4)	0.40
Male, n (%)	8 (72.7)	5 (55.6)	
Age, years	28 (19-36)	20 (18-35)	0.20
Training time, hours/week	7.5 (3-10)	15 (15-15)	0.60

Data are presented as median and range unless otherwise specified. NHM: non-hypermobile, IHM: increased mobility and hypermobility

There were no significant differences when comparing expression levels of the β-Actin gene (p> 0.05). Since the genes of interest were not expressed at high levels in both groups, they were not quantified after cycles. No significant differences were found in the relative gene expressions of COL1A1, COL5A1, or COL12A1 between the groups.

DISCUSSION

An excess of flexibility, as GJL, is a heritable connective tissue disorder, in which joints have a range of motion exceeding the normal range (14). Although this high level of flexibility is a desirable feature in particular sports where it is particularly important for success, GJL is known to be an internal risk factor for especially tendon and ligament injuries (24). In this study, the expression levels of COL1A1, COL5A1, and COL12A1 genes that affect the structure of connective tissue in participants with and without GJL were investigated. We found no significant differences in the relative gene expressions of COL1A1, COL5A1, or COL12A1 between the NHM and the IHM groups.

Wang et al. examined the messenger RNA (mRNA) expression of COL1A1 in the hip capsule of children with developmental hip dislocation to investigate the relation between collagen type I and hip joint laxity (25). They found that mRNA expression of COL1A1 in the developmental dislocation of the hip group was significantly lower than that in the control group. The authors stated that the location of the COL1A1 gene in relating region might be the reason for this relationship. In a recent study, Tuna et al. examined Ehlers-Danlos syndrome-related gene expressions from blood samples in generalized joint hypermobility (26). Similar to the study of Wang et al., they revealed that COL1A1 and COL5A1 gene expressions were lower in the generalized joint hypermobility group.

In a case-control study, Rouault et al. investigated the association between COL1A1 polymorphisms and congenital dislocation of the hip that is also linked to capsular joint laxity (27). In contrary to the abovementioned studies, their results did not support any association between

COL1A1 and congenital dislocation of the hip in their population.

Studies demonstrated an association between the COL1A1, COL5A1, and COL12A1 gene variants and ACL injury (4,5,9,13). It is known that increased magnitudes of anterior knee laxity, genu recurvatum, and GJL have been consistently associated with a greater risk of ACL injury (28,29). In the light of this information, Bell et al. investigated the genetic variants within the COL1A1, COL5A1, and COL12A1 genes, which were previously associated with ACL injury, in participants with anterior knee laxity, genu recurvatum, and GJL (20). The authors examined single nucleotide polymorphisms that were previously associated with ACL damage. They found that specific genotypes were associated with greater genu recurvatum in all subjects, whereas some genotypes were associated with greater magnitudes of genu recurvatum, anterior knee laxity, and GJL in females only. Moreover, they observed no association with single-nucleotide polymorphisms that are not associated with ACL injury. The authors explained this result as such that specific genetic changes in collagen genes would increase ACL injury risk by altering the amount and structure of collagen proteins. This may also explain our results displaying that expressions of collagen genes were similar in individuals with and without hypermobility.

In our study, we could not quantify the expression levels for genes of interest. This might be explained by the fact that when the mRNA quantity of the gene does not exceed a detection threshold, the corresponding Cycles to Threshold value is undetermined or close to the upper limit of the possible range (30,31). In such cases, it is recommended that the detector should be considered "not detected" (31). Therefore, it was concluded that our study groups were not different from each other.

Our study has some limitations. The primary limitation is the low number of participants. Studies with more participants may provide us more comprehensive information. Our second limitation is that we only examined the gene expressions in our study, but not the single-nucleotide polymorphisms. Genome-wide studies are recommended to evaluate the more potential genetic variants related to GJL.

CONCLUSION

The gene expression levels of collagen types I, III, and V in the blood of participants with and without GJL were not different. Genome-wide studies are recommended to evaluate the potential genetic variants associated with hypermobility, which may relate with sport injuries.

Conflict of Interest

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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REFERENCES

1. Lippi G, Longo UG, Maffulli N. Genetics and sports. *Br Med Bull*. 2010;93:27–47.
2. Rossert J, Terraz C, Dupont S. Regulation of type I collagen genes expression. *Nephrol Dial Transplant*. 2000;15 Suppl 6:66–8.
3. Hoffmann A, Gross G. Tendon and ligament engineering in the adult organism: mesenchymal stem cells and gene-therapeutic approaches. *Int Orthop*. 2007;31(6):791–7.
4. Khoschnau S, Melhus H, Jacobson A, Rahme H, Bengtsson H, Ribom E, Grundberg E, Mallmin H, Michaëlsson K. Type I collagen alpha1 Sp1 polymorphism and the risk of cruciate ligament ruptures or shoulder dislocations. *Am J Sports Med.* 2008;36(12):2432–6.
5. Posthumus M, September A V, Keegan M, O'Cuinneagain D, Van der Merwe W, Schwellnus MP, Collins M. Genetic risk factors for anterior cruciate ligament ruptures: COL1A1 gene variant. *Br J Sports Med*. 2009;43(5):352–6.
6. Hildebrand KA, Frank CB, Hart DA. Gene intervention in ligament and tendon: current status, challenges, future directions. *Gene Ther.* 2004;11(4):368–78.
7. Longo UG, Fazio V, Poeta ML, Rabitti C, Franceschi F, Maffulli N, Denaro V. Bilateral consecutive rupture of the quadriceps tendon in a man with BstUI polymorphism of the COL5A1 gene. *Knee Surg Sports Traumatol Arthrosc.* 2010;18(4):514–8.
8. Mokone GG, Schwellnus MP, Noakes TD, Collins M. The COL5A1 gene and Achilles tendon pathology. *Scand J Med Sci Sports*. 2006;16(1):19–26.
9. Posthumus M, September A V, O'Cuinneagain D, van der Merwe W, Schwellnus MP, Collins M. The COL5A1 gene is associated with increased risk of anterior

cruciate ligament ruptures in female participants. *Am J Sports Med.* 2009;37(11):2234–40.
10. September A V, Cook J, Handley CJ, van der Merwe L, Schwellnus MP, Collins M. Variants within the COL5A1 gene are associated with Achilles tendinopathy in two populations. *Br J Sports Med.* 2009;43(5):357–65.
11. Shaw LM, Olsen BR. FACIT collagens: diverse molecular bridges in extracellular matrices. *Trends Biochem Sci.* 1991;16(5):191–4.
12. Young BB, Zhang G, Koch M, Birk DE. The roles of types XII and XIV collagen in fibrillogenesis and matrix assembly in the developing cornea. *J Cell Biochem*. 2002;87(2):208–20.
13. Posthumus M, September A V, O'Cuinneagain D, van der Merwe W, Schwellnus MP, Collins M. The association between the COL12A1 gene and anterior cruciate ligament ruptures. *Br J Sports Med.* 2010;44(16):1160–5.
14. Hakim A, Grahame R. Joint hypermobility. *Best Pract Res Clin Rheumatol.* 2003;17(6):989–1004.
15. Beighton PH, Horan FT. Dominant inheritance in familial generalised articular hypermobility. *J Bone Joint Surg Br.* 1970;52(1):145–7.
16. Bridges AJ, Smith E, Reid J. Joint hypermobility in adults referred to rheumatology clinics. *Ann Rheum Dis*. 1992;51(6):793–6.
17. Dowdy PA, O'Driscoll SW. Recurrent anterior shoulder instability. *Am J Sports Med*. 22(4):489–92.
18. Silman AJ, Day SJ, Haskard DO. Factors associated with joint mobility in an adolescent population. *Ann Rheum Dis*. 1987;46(3):209–12.
19. Hakim AJ, Cherkas LF, Grahame R, Spector TD, MacGregor AJ. The genetic epidemiology of joint hypermobility: a population study of female twins. *Arthritis Rheum.* 2004;50(8):2640–4.
20. Bell RD, Shultz SJ, Wideman L, Henrich VC. Collagen gene variants previously associated with anterior cruciate ligament injury risk are also associated with joint laxity. *Sports Health*. 2012;4(4):312–8.
21. Baeza-Velasco C, Gély-Nargeot M-C, Pailhez G, Vilarrasa AB. Joint Hypermobility and Sport. *Curr Sports Med Rep*. 2013;12(5):291–5.
22. Beighton P, Solomon L, Soskolne CL. Articular mobility in an African population. *Ann Rheum Dis*. 1973;32(5):413–8.
23. Boyle KL, Witt P, Riegger-Krugh C. Intrarater and Interrater Reliability of the Beighton and Horan Joint Mobility Index. *J Athl Train.* 2003;38(4):281–5.
24. McCormack M, Bird H, de Medici A, Haddad F, Simmonds J. The Physical Attributes Most Required in Professional Ballet: A Delphi Study. *Sport Med Int Open*. 2019;03(01):E1–5.
25. Wang E-B, Zhao Q, Li L-Y, Shi L-W, Gao H. [Expression of COL1a1 and COL3a1 in the capsule of children with developmental dislocation of the hip]. *Zhongguo Dang Dai Er Ke Za Zhi*. 2008;10(4):493–6.
26. Tuna F, Doğanlar ZB, Özdemir H, Demirbag Kabayel D, Doğanlar O. Ehlers–Danlos syndrome-related genes and serum strontium, zinc, and lithium levels in generalized joint hypermobility: a case-control study. *Connect Tissue Res.* 2019;1–11.
27. Rouault K, Scotet V, Autret S, Gaucher F, Dubrana F, Tanguy D, Yaacoub El Rassi C, Fenoll B, Férec C. Do HOXB9 and COL1A1 genes play a role in congenital dislocation of the hip? Study in a Caucasian population. *Osteoarthr Cartil.* 2009;17(8):1099–105.
28. Kramer LC, Denegar CR, Buckley WE, Hertel J. Factors associated with anterior cruciate ligament injury: History in female athletes. *J Sports Med Phys Fitness.* 2007;47(4):446–54.
29. Scerpella TA, Stayer TJ, Makhuli BZ. Ligamentous laxity and non-contact anterior cruciate ligament tears: A gender-based comparison. *Orthopedics.* 2005;28(7):656–60.
30. Nolan T, Hands RE, Bustin SA. Quantification of mRNA using real-time RT-PCR. *Nat Protoc.* 2006;1(3): 1559-82.
31. Goni R, García P, Foissac S. The qPCR data statistical analysis. Integromics White Paper. 2009;1–9.