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Determination of *COL1A1* rs1800012 Polymorphism Related to Ligament and Tendon Injury in Turkish Professional Bodybuilders: Experimental Research

Türk Profesyonel Vücut Geliştirmecilerde Bağ ve Tendon Yaralanmalarına İlişkin *COL1A1* rs1800012 Polimorfizminin Belirlenmesi: Deneysel Araştırma

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ABSTRACT Objective: Athletic performance is affected by genetic and environmental factors. Bodybuilding has been increasing around the world in recent years and it is accepted that the factors affecting athletic performance are the most important influences to achieve success in bodybuilding. The aim of our study is to determine the distribution of *COL1A1* rs1800012 polymorphism associated with ligament and tendon injuries due to muscle tears with weight training and to compare the results with sedentary individuals. **Material and Methods:** A total of 15 Turkish bodybuilders and 50 sedentary individuals as a control group participated in our study. DNA isolation from peripheral blood samples of athletes was performed using a commercial kit (Invitrogen, Van Allen Way Carlsbad, Calif., USA). Genotyping was done by real-time polymerase chain reaction. **Results:** GG genotype and G allele were higher in both bodybuilders and control groups. In addition, there was no statistically significant difference in terms of genotype and allelic frequency of the *COL1A1* rs1800012 polymorphism between the bodybuilders and the control group. **Conclusion:** Our study is the first to examine the *COL1A1* rs1800012 polymorphism in Turkish bodybuilders. Our results report that the TT genotype and T allele may be protective against ligament and tendon injuries, similar to the literature. We think that similar studies will guide future studies in terms of sports injuries and *COL1A1* rs1800012 polymorphism.

Keywords: Bodybuilder; *COL1A1*; injuries risk; polymorphisms; sports genetics

ÖZET Amaç: Atletik performans, genetik ve çevresel faktörlerden etkilenir. Vücut geliştirme, son yıllarda tüm dünyada artış göstermekte ve vücut geliştirmede başarıya ulaşmada atletik performansı etkileyen faktörlerin en önemli etkenler olduğu kabul edilmektedir. Çalışmamızın amacı, ağırlık antrenmanı ile kas yırtılmalarına bağlı bağ ve tendon yaralanmalarına ait *COL1A1* rs1800012 polimorfizminin dağılımını belirlemek ve sonuçları sedanter bireyler ile karşılaştırmaktır. **Gereç ve Yöntemler:** Çalışmamıza kontrol grubu olarak toplam 15 Türk vücut geliştirmeci ve 50 sedanter birey katıldı. Sporcuların periferik kan örneklerinden DNA izolasyonu ticari bir kit (Invitrogen, Van Allen Way Carlsbad, Calif., ABD) kullanılarak yapıldı. Genotipleme, gerçek zamanlı polimeraz zincir reaksiyonu ile yapıldı. **Bulgular:** GG genotipi ve G aleli hem vücut geliştirmecilerde hem de kontrol gruplarında daha yüksekti. Ayrıca vücut geliştiriciler ile kontrol grubu arasında *COL1A1* rs1800012 polimorfizminde genotip ve alelik frekans açısından istatistiksel olarak anlamlı fark yoktu. **Sonuç:** Çalışmamız, Türk vücut geliştirme sporcularında *COL1A1* rs1800012 polimorfizmini inceleyen ilk çalışmadır. Sonuçlarımız literatüre benzer şekilde TT genotipi ve T alelinin bağ ve tendon yaralanmalarına karşı koruyucu olabileceğini bildirmektedir. Spor yaralanmaları ve *COL1A1* rs1800012 polimorfizmi açısından benzer çalışmaların ileride yapılacak çalışmalara yön vereceğini düşünüyoruz.

Anahtar Kelimeler: Vücut geliştirmeci; *COL1A1*; yaralanma riski; polimorfizmler; spor genetiği

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Athletic performance is one of the power limitations of our body and is considered to be affected by genetic and environmental factors. Current sports genetics studies focus on genetic variants that can affect athletic performance. Studies reported the genetic background of factors affecting athletic performance like endurance, power, muscle coordination, and also the effect of psychological factors and nutrition in athletic performance.[1-4] Competitive and performance athletes come across acute injuries, especially tendons, and ligaments, and these injuries constitute the most.[5] To prevent injuries, genetic data provide valuable outputs to set personal training sessions which is more compatible with the athlete's physiology. Also threat about gene doping is still on the table in which athletes can undergo to improve their athletic performance.[6-8]

Bodybuilding is an activity performed by individuals who aim to achieve a symmetrical and proportional appearance by acquiring body shape and muscle mass. In the last few years, bodybuilding has become one of the world's most popular sports. This sport, which is common in many countries, provides a healthy life for people in many ways such as physical therapy, fitness, weight loss, and empowerment. Bodybuilders undergo a combination of strength training, optimal nutrition, and resting times for the best muscle hypertrophy. The most important activity for achieving success in bodybuilding is accepted to be the weight training sections, in which they use various weights, such as dumbbells and barbells, different training sets, as well as calorie intake, to improve muscle fibers. Aerobic and anaerobic exercise sets are used in weight training sections, and at the same time, it is a kind of recreational sport.[9]

Type 1 collagen is an important component of bones and an abundant structural protein in cartilage, ligaments, tendons, blood vessels, and the outer cell matrix of the subcutaneous tissue. Type 1 collagen almost represents 80% of the dry weight of tendons and ligaments and is composed of two chains: α1 and α2, at a ratio of 2:1.[10] The collagen Type 1 alpha-1 (*COL1A1*) gene is located on chromosome 17q21.33 and is responsible for the synthesis of the pro-alpha-1 chain. Mutations in the *COL1A1* gene may cause certain types of hereditary diseases, and mutations very close to the gene can also cause osteogenesis imperfecta/Ehlers-Danlos syndrome.[11] *COL1A1* has a functional polymorphism, rs1800012, a G/T transversion at Sp1 binding site in the 1st intron of the gene. The *COL1A1* rs1800012 polymorphism is the most frequently studied polymorphism in this gene region and has been associated with an increased risk of acute sports-related musculoskeletal soft tissue injuries.[5,12]

In the present study, we aimed to determine the distribution of *COL1A1* rs1800012 polymorphism genotype and alleles in professional bodybuilders and compare the results with sedentary individuals.

MATERIAL AND METHODS

PARTICIPANTS

Fifteen professional male bodybuilders were enrolled in the study. Six of them had a national status in their sports life, and one of them had the silver medal in a national competition and represented the Turkish National team in European competitions. Besides, all the participants had at least 6 sessions per week and a total of approximately 10 hours/week of training sessions. In addition, 50 sedentary individuals who did not do sports regularly participated in our study as a control group. Consent forms containing all information such as the study protocol and results were signed by both the athletes and the control group before the study.

RESEARCH ETHICS

The study was approved by the Üsküdar University Non-Invasive Research Ethics Committee (date: March 30, 2023; no: 61351342/03.2023-48).

GENOTYPING

Peripheral blood samples were collected by signing prospective forms from all participants. Study protocols and experiment procedures were carried out according to the Helsinki Declaration II principles. DNA isolation was carried out by using a commercially available PureLink DNA Isolation Kit (Invitrogen, Van Allen Way Carlsbad, Calif., USA). The operations were carried out with respect to the manufacturer's instructions. Briefly, 20 µL proteinase K was vortexed by adding 10µL of RNAase to 200 µL

of blood samples. 200µL of binding buffer was added and homogenized by slowly stirring. After incubation for 10 minutes at 55°C, 200 µL ethanol was added and vortexed for 5 seconds. It was taken to the filtered tube and centrifuged at 10,000 g for 1 minute. The supernatant was discarded and 500 µL of washing buffer was added to the pellet and centrifuged at 10,000 g for 1.15 seconds. 80 µL of elution buffer was added and incubated and centrifuged at maximum speed for 1 minute.

All the genotyping procedures were carried out by real-time polymerase chain reaction (Quant Studio-3, Termofisher, USA). For genotyping process, *COL1A1* TaqMan SNP Genotyping Assay (Termofisher, USA) was used by following the manufacturer's guide.[13] Genotyping was completed using 5 µL master mix, 3.75 µL H_2O, 0.25µL assay, and 1µL (10 ng) DNA.

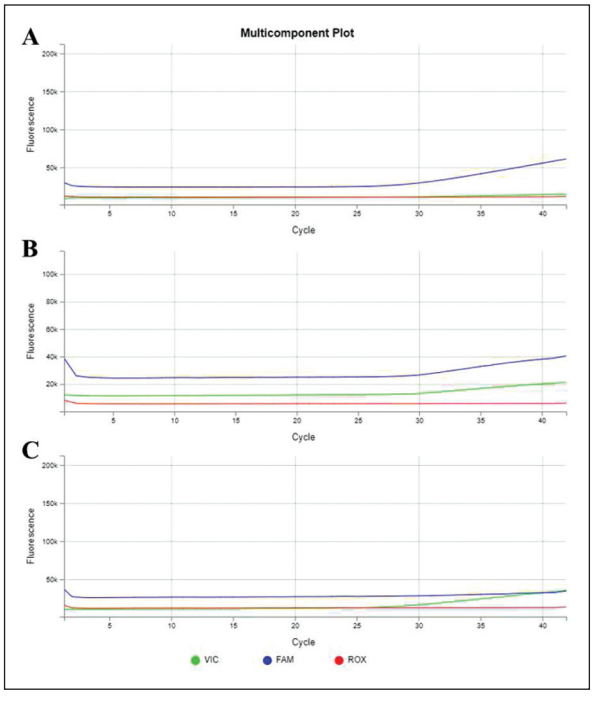

FIGURE 1: Multicomponent Plot images in real-time polymerase chain reaction of the GG, GT, and TT genotypes of the COL1A1 rs1800012 polymorphism. The G allele (blue curve) is indicated by the FAM dye, while the T allele (green curve) is indicated by the VIC dye. (A) GG genotype is shown with a single blue curve, (B) GT genotype is shown with both blue and green curves, and (C) TT genotype is shown with a single green curve.

The G allele for the FAM primer and the T allele for the VIC primers were used for the detection of *COL1A1* rs1800012 polymorphism (Figure 1). The TaqMan Probe sequences used for genotyping are shown in Table 1.

STATISTICAL ANALYSIS

The genotype distribution and allele frequency between the athlete group and the control group obtained using the SPSS (version 25.0 for Windows, SPSS, Chicago, IL, USA) program were compared with the χ^2 test. $p<0.05$ value was accepted as statistically significant.

RESULTS

The numbers and percentages of GG, GT, and TT genotypes for bodybuilders (n=15) were 10 (66.7%), 3 (20%), and 2 (13.3%), respectively. The G allele (76.7%) was counted by a percentage higher than the T allele (23.3%). The numbers and percentages of GG, GT, and TT genotypes for the control group (n=50) were 26 (52.0%), 18 (36.0%), and 6 (12.0%), respectively. In the control group, the G allele (70.0%) was counted by a percentage higher than the T allele (30.0%). In the statistical analysis between bodybuilders and control groups, no statistically significant difference was found in terms of both genotype distribution (p=0.5018) and allelic frequency (p=0.4779). All results are listed in Table 2.

TABLE 1: Sequences of the TaqMan probe used for genotyping *COL1A1* rs1800012 polymorphism

qPCR	Sequence, 5'-3'
VIC/FAM	GGGAGGTCCAGCCCTCATCCCGCCC(A/C)CATTCCCTGGGCAGGTG GGGTGGCG

PCR: Polymerase chain reaction.

TABLE 2: Genotype distribution and allele frequency of *COL1A1* rs1800012 polymorphism in bodybuilders.

	Genotype				Allelic frequency		
	GG	GT	TT	p value	G	T	p value
Bodybuilders (n=15)	10	3	2	0.50*	23	7	0.47*
Percentage	66.7%	20.0%	13.3%		76.7%	23.3%	
Control group (n=50)	26	18	6		70	30	
Percentage	52.0%	36.0%	12.0%		70.0%	30.0%	

*Significance was assessed at least at the p<0.05 level. Comparison with the control group was made using the χ^2 test.

DISCUSSION

Having the optimal structural conditions for an athlete is one of the most important factors affecting athletic performance. Weight training may cause tears in muscles, and biological factors are important for the healing of muscles. As bodybuilding depends on functional muscle movement, metabolism, and muscle-supporting structures, tendons and ligaments are the most important support structures for optimal exercise conditions in terms of bodybuilders. In this regard, the T allele of rs1800012 polymorphism of *COL1A1* was before considered to have an effective role for sports-related tendon and ligament injuries due to the high transcription activity.

In our cohort, the GG genotype (66.7%) was found to be a percentage higher than GT and TT genotypes in bodybuilders. At the same time, when we compared the alleles, the percentage of the G allele (76.7%) was detected higher than the T allele. Our findings are similar to those of previous studies.

Khoschnau et al. analyzed 233 people with anterior cruciate ligament (ACL) rupture and 126 people with a shoulder dislocation and reported that they found the GG genotype and G allele to be higher in both groups, whereas they found only one with the TT genotype.[14] Like this study, Posthumus et al. reported the absence of TT genotypes in 117 individuals who had ACL ruptures.[15] Similar findings were reported before, authors detected no TT genotypes in 41 individuals with Achilles tendon ruptures. The same study reported only 2 individuals with TT genotypes in 85 individuals with chronic Achilles tendinopathy.[16] In the present study, due to the high percentage of the GG genotype, it can be speculated that the GG genotype and the G allele may have some advantages for body-building activities.

Ficek et al. analyzed 91 football players and compared them with 143 sedentary controls in terms of *COL1A1* rs1107946 and rs1800012 polymorphisms in ACL ruptures.[17] According to their findings, the TT genotype is less represented in ruptured players and the G-T haplogroup of both polymorphisms was found to be higher in healthy subjects. These findings showed the protective role of the examined polymorphism in players. In a similar study, Stępien-Słodkowska et al. examined the rs1800012 polymorphism in the *COL1A1* gene in 138 Polish male recreational skiers with ACL ruptures, and they reported that the risk of ACL ruptures was approximately 1.43 times lower in carriers of the T allele compared to the G allele.[18]

Dines et al. examined the *COL1A1* rs1800012 and *COL5A1* rs12722 polymorphisms in a total of 1,429 helmet subjects, including 597 competitive runners (354 males and 243 females) and 832 non-athletes (490 males and 342 females). They reported that competitive runners were approximately 2 times more likely than non-athletes to have a combination of the injury-resistant *COL1A1* rs1800012 TT and *COL5A1* rs12722 CC genotypes.[19]

Jacob et al., examined the polymorphisms in *ACTN3*, *CCL2*, *COL1A1*, *COL5A1*, *COL12A1*, *EMILIN1*, *IGF2*, *NOGGIN*, *SMAD6* genes associated with injury in 46 elite male Australian football players.[20] As a result different from the literature, they found a significant relationship between low-severity ligament-related injuries and *COL1A1* rs1800012

TT genotype. However, the contradictory results of their study reported that the GG genotype may be underrepresented in the current population.

When the studies on the genotype and allele frequency distributions of the *COL1A1* rs1800012 polymorphism in the Turkish population were examined; Erduran et al. on tennis players, Aslan et al. on cyclists, and Bulğay et al. on long-distance and short-distance runners had similar findings, they all reported that the TT genotype and T allele distribution ratio were underrepresented in the population compared to the GG genotype and G allele.[13,21,22]

To date, studies on bodybuilders are limited. Polat et al. reported that *ACTN3* CT genotype and C allele and *ACE* ID genotype and I allele were observed more frequently when they examined *ACE* InDel and *ACTN3* rs1815739 polymorphisms in Turkish bodybuilders.[23] This illustrates the importance of the endurance-related *ACE* and *ACTN3* alleles in bodybuilders.

The low number of subjects was the main limitation of the study. We surveyed a number of bodybuilders and we agreed to enroll 15 of them who had our criteria. The second limitation of the study is not having detailed injury records of the bodybuilders. During their sports activities, they reported several injuries, but all of them are not supported by medical findings. Most of them had some injuries but did not have detailed medical reports. When we searched the literature, we also had some restricted articles about body-builders and *COL1A1* polymorphisms.

CONCLUSION

Genes are very important in determining athletic performance, and also in injury prevention. As athletic performance involves multifactorial elements, analyzing the gene groups instead of single genes may provide more precise results. Our study is the first to examine the distribution of the *COL1A1* rs1800012 polymorphism in Turkish bodybuilders. In our cohort, results revealed that the TT genotype and T allele may be protective against tendon and ligament injuries and better response to training, similar to previous studies.

But, the unclarity of enough information of *COL1A1* rs1800012 polymorphism, as well as the limited number of studies including body-building athletes and lacking the knowledge of injuries proven by medical records stand out as limiting factors in the interpretation of the outcomes of the study. Within this framework, we suggest the present study as to contribute to the developments in the field of sports sciences along with the limited number of other similar studies.

In addition, we think that this study will lead to future studies in terms of sports injuries and *COL1A1* rs1800012 polymorphism.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Özlem Özge Yılmaz, Tolga Polat, Beste Tacal Aslan, Raif Zileli, Korkut Ulucan;* ***Design:*** *Özlem Özge Yılmaz, Tolga Polat, Beste Tacal Aslan, Raif Zileli, Korkut Ulucan;* ***Control/Supervision:*** *Özlem Özge Yılmaz, Tolga Polat, Beste Tacal Aslan, Raif Zileli, Korkut Ulucan;* ***Data Collection and/or Processing:*** *Özlem Özge Yılmaz, Tolga Polat, Beste Tacal Aslan, Raif Zileli, Korkut Ulucan, İpek Yüksel Gözler, Kubilay Göçücü;* ***Analysis and/or Interpretation:*** *Beste Tacal Aslan, Raif Zileli, Korkut Ulucan, Özlem Özge Yılmaz, Tolga Polat;* ***Literature Review:*** *Özlem Özge Yılmaz, Tolga Polat, Beste Tacal Aslan, Raif Zileli, Korkut Ulucan;* ***Writing the Article:*** *Özlem Özge Yılmaz, Tolga Polat, Beste Tacal Aslan, Raif Zileli, Korkut Ulucan;* ***Critical Review:*** *Beste Tacal Aslan, Raif Zileli, Korkut Ulucan;* ***References and Fundings:*** *Özlem Özge Yılmaz, Tolga Polat, Beste Tacal Aslan, Raif Zileli, Korkut Ulucan;* ***Materials:*** *Özlem Özge Yılmaz, Tolga Polat, Beste Tacal Aslan, Raif Zileli, Korkut Ulucan.*

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