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Investigation of the *ACTN3* Gene Polymorphism (rs1815739) and FMS Values in Young Runners: Cross-Sectional Research

Genç Koşucularda *ACTN3* Gen Polimorfizmi (rs1815739) ile FMS Değerlerinin İncelenmesi: Kesitsel Araştırma

Celal BULGAY[a], Işık BAYRAKTAR[b], Meriç ÖDEMİŞ[b], Damla Selin YILDIRIM[c], Mehmet Ali ERGÜN[d], Mesut CERİT[c], Korkut ULUCAN[e]

[a]Department of Physical Education and Sports Teaching, Bingöl University Faculty of Sport Sciences, Bingöl, Türkiye
[b]Department of Coaching Education, Alanya Alaaddin Keykubat University Faculty of Sport Sciences, Alanya, Türkiye
[c]Department of Coaching Education, Lokman Hekim University Faculty of Sport Sciences, Ankara, Türkiye
[d]Department of Medical Genetics, Gazi University Faculty of Medicine, Ankara, Türkiye
[e]Department of Medical Biology and Genetics, Marmara University, İstanbul, Türkiye

ABSTRACT Objective: The present study aims to examine investigation the α-actinin-3 (ACTN3) gene polymorphism (rs1815739) and functional movement screen (FMS) values in young runners competing at the national level. **Material and Methods:** Forty-five young runners (29 male 64.4%, 16 female 35.6%) competing at the national level in different clubs affiliated to the Turkish Athletic Federation and training regularly at least 5 days a week voluntarily participated in the study. The DNA isolation of oral swab samples was carried out using the spin column (MN Macherey-Nagel, Germany) method. The genotyping process was performed using the real-time polymerase chain reaction method. **Results:** The FMS test battery was used to determine the functional capacity. According to the findings obtained, it was determined that in the FMS evaluation of the female runners, the runners with the CC genotype had a significantly higher mean score compared to the runners with the CT genotype ($p<0.033$) while in the male runners, the runners with the CT genotype had a significantly higher mean score compared to the runners with the TT genotype ($p<0.031$). However, in the FMS evaluation of the overall runners, no significant difference was found ($p>0.205$). **Conclusion:** It is thought that the FMS test battery and the *ACTN3* gene polymorphism, which is among the important variables responsible for non-impact injuries, can be effective in the level of muscle damage, the type of muscle injury, the prediction of injuries and reducing the risk factors that may cause injuries.

Keywords: *ACTN3*; athletic performance; FMS; runners; risk of injury; polymorphism rs1815739

ÖZET Amaç: Sunulan çalışma, ulusal düzeyde yarışan genç koşucularda α-aktinin-3 [α-actinin-3 (ACTN3)] gen polimorfizmi (rs1815739) ve fonksiyonel hareket analizi [functional movement screen (FMS)] değerlerini incelemeyi amaçlamaktadır. **Gereç ve Yöntemler:** Araştırmaya, Türkiye Atletizm Federasyonuna bağlı farklı kulüplerde lisanslı olarak ulusal düzeyde yarışan, haftada en az 5 gün düzenli antrenman yapan 45 genç koşucu (29'u erkek %64,4, 16'sı kadın %35,6) gönüllü olarak katılmıştır. Ağız sürüntü örneklerinin DNA izolasyonu spin kolon (MN Macherey-Nagel, Almanya) yöntemi ile gerçekleştirilmiştir. Genotipleme işlemi gerçek zamanlı polimeraz zincir reaksiyonu metodu ile gerçekleştirilmiştir. Fonksiyonel kapasitenin belirlenmesi için FMS test bataryası kullanılmıştır. **Bulgular:** Elde edilen bulgulara göre kadın koşucuların FMS değerlendirmesinde, CC genotipe sahip koşucuların ortalama puanının CT genotipe sahip koşucular dan ($p<0,023$), erkek koşucularda ise CT genotipe sahip koşucuların ortalama puanı TT genotipe sahip koşucular dan ($p<0,13$) anlamlı bir şekilde daha yüksek olduğu tespit edilmiştir. Ancak toplam koşucuların FMS değerlendirmesinde genotipe göre anlamlı bir farklılık tespit edilmemiştir ($p>0,145$). **Sonuç:** Darbelere bağlı olmayan yaralanmalardan sorumlu önemli değişkenlerden *ACTN3* gen polimorfizmi ve FMS test bataryasının, koşucularda kas hasarı düzeyine, kas yaralanma tipine, yaralanmaların önceden tahmin edilmesine ve yaralanmalara neden olabilecek risk faktörlerinin azaltılmasına etkili olacağı düşünülmektedir.

Anahtar Kelimeler: ACTN3; atletik performans; FMS; koşucular; yaralanma riski; polimorfizmi rs1815739

Running is one of the most popular sports branches in the world, attracting the interest of hundreds of thousands of people of all ages.[1] The main reason behind this is regarded as the accessibility, low-cost and ease of application as well as the physiological and psychological benefits of running.[2]

Correspondence: Celal BULGAY
Department of Physical Education and Sports Teaching, Bingöl University Faculty of Sport Sciences, Bingöl, Türkiye
E-mail: celalbulgay@hotmail.com

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However, the increasing popularity of running can also cause an increase in the number of injuries related to running. This increase stands out as an important problem in terms of both the health and performance of runners. In addition to these, in cases where competitive long-distance runs, intense training, frequent competition and other similar loads are increased, athletes can be exposed to injury and diseases. When the risk of injury in runners is evaluated, it is known that approximately 50% of athletes suffer more than one injury each year. Although running-induced injuries are affected by countless factors, the age, training volume, history of previous injuries and running kinematics of athletes are regarded as the main determinants of the likelihood of suffering injuries related to running.[3]

In previous studies, it was reported that in order to reach high performance levels or protect themselves from non-impact injuries, it is necessary for runners to utilize the results of tests in which genetic factors are determined in addition to field and laboratory tests.[4] One of the polymorphisms that are thought to be related to non-impact injury is the alpha-actinin-3 (*ACTN3*) gene.[5] The *ACTN3* gene is a 901-amino-acid gene found in the q13.1 region of the long arm of chromosome 11 that codes for the ACTN3 protein in our genome, which forms the thin muscle fiber in the sarcomere muscle unit.[6] The *ACTN3* polymorphism, which is a determining factor for high-speed muscular contraction and high power generation, is categorized as CC (fast-twitch fiber type b), CT (fast-twitch fiber type a) and TT (slow-twitch fiber) genotypes. When the related literature is reviewed, it is observed that the *ACTN3* gene polymorphism is related to not only athletic performance, but also injury. For example, it was revealed that the skeletal muscles of individuals with the *ACTN3* XX genotype are more frequently exposed to damage related to traumatic, toxic and metabolic factors compared to those with the RR genotype. In other words, it was reported that athletes with the *ACTN3* XX genotype are more susceptible to certain non-impact injuries such as muscle injury, ankle sprains and exercise-induced muscle damage.[8] When the impact of the *ACTN3 R577X* gene polymorphism on muscle damage in athletes competing in ultramarathons was investigated, it was determined that athletes with the XX genotype suffered more muscle damage compared to athletes with the RR genotype according to the findings obtained after a competition.[5] In a similar study conducted with football players, it was determined that footballers with the XX genotype were more susceptible to non-contact musculoskeletal soft tissue injuries compared to footballers with the RR or RX genotypes.[9] In contrast with these findings, in a study conducted on Japanese female athletes (football, volleyball, basketball and badminton), it was found that athletes with the RR genotype suffered more severe muscle damage compared to athletes with the XX genotype.[10]

Branch selection and intense training may affect the musculoskeletal status of athletes in the lower age category and cause them to get injured. Potential sports injuries in this age group can be predicted beforehand.[11] Certain studies reported that the functional movement screen (FMS) has the ability to predict injury-prone individuals.[12,13] FMS takes many functional movement patterns necessary in athletics into account and constitutes an auxiliary element to determine potential injury risks.[14] When previous studies utilizing the FMS test battery are examined, it was reported that athletes with a mean score of 14 or less on the FMS were 15 times more likely to get injured compared to athletes that scored higher on the FMS.[14] Another study supporting this was conducted on professional football players. It was found that footballers with a mean score of 14 on the FMS were at risk of suffering more severe injury compared to athletes with higher scores.[15] Another study on various sports branches was conducted by Shojaedin et al. According to the results obtained, an odds ratio was calculated at 4.70, meaning that an athlete has an approximately 4.7 times greater chance of suffering a lower extremity injury during a regular competitive season if they score less than 17 on the FMS.[16] In the context of previous studies carried out with different populations and groups, it can be said that individuals having the TT genotype or the T allele and low score FMS is linked with injury. However, due to the limitations of previous studies, the effect mechanism of the rs1815739 polymorphism on injury could not be fully clarified.

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Therefore, it was stated that possessing field and laboratory findings in addition to the genotype analyses of athletes may be necessary in order to detail the mechanism of action of non-impact injuries in runners, achieve long-term performance development and minimize the risk of injury.[17] Both *ACTN3* and the FMS test battery are important factors that need to be carefully examined in order to predict potential injuries beforehand, reduce the risk factors that may cause injuries and prevent the recurrence of injuries. It is clear that not only *ACTN3*, but also FMS findings are needed to better understand the relationship between non-impact injuries in runners. In this context, the present study aims to examine the *ACTN3* gene polymorphism and FMS values in young runners competing at the national level. We hypothesized that female, male and overall runners with TT genotype would be present with lower scores on the FMS.

MATERIAL AND METHODS

ETHICS COMMITTEE APPROVAL

Prior to its initiation, the study was evaluated by Lokman Hekim University Ethics Committee and approved (date: May 31, 2022, no: 2022/9-1). Additionally, the present study was prepared in accordance with the Declaration of Helsinki protocol of principles.

STUDY GROUP

Forty-five young runners (29 male 64.4%, 16 female 35.6%; $\bar{X}_{age\ (years)}$=14.50, SD 1.43; $\bar{X}_{height\ (cm)}$=165.49, SD 8.18; $\bar{X}_{weight\ (kg)}$=47.78, SD 8.33; $\bar{X}_{sports\ experience\ (years)}$=3.08, SD 1.65; X_{FMS}=16.57, SD 1.75; Distance: Female (1,500 m-2,000 m), male (2,000-3,000 m) competing at the national level in different clubs affiliated to the Turkish Athletic Federation and training regularly at least 5 days a week voluntarily participated in the study.

APPLICATION PHASE

Forty-five male and female young runners (participants) aged 12-17 voluntarily participated in the present study. The participants were informed regarding the measurement procedure and the study 1 week prior to the start of the study. Before the measurements, informed consent and demographic information forms were collected from the participant groups. In general, the participants took part in the test using the sports equipment they use in training. After the participants were informed about the FMS test battery, scoring was performed through a simultaneous evaluation by a physiotherapist and a sports scientist, and the scores were analyzed afterwards.[20] In the study, oral swab samples for the *ACTN3* gene polymorphism analysis were taken from all groups 2 times. The measurements of the study were performed in cooperation with the hospital where the participants carried out their routine controls during the preparation period. The oral swab samples taken from the athletes were taken to Gazi University Medical Genetics Laboratory. The DNA isolations of the samples sent to the laboratory were carried out. rs1815739 polymorphism in the *ACTN3* gene were studied using real-time polymerase chain reaction (PCR). All molecular analyses were performed in Gazi University Medical Genetics Laboratory.

DATA COLLECTION TOOLS

ACTN3 rs1815739 Genotyping

The DNA isolation of oral swab samples was carried out using the spin column (MN Macherey-Nagel, Germany) method. The DNA samples isolated were measured in a spectrophotometer (NanoDrop, ND 1000, USA). Afterwards, the DNA samples were placed in a freezer (-20°C). The DNA samples obtained were both evaluated in an agarose gel electrophoresis and measured in a spectrophotometer to determine their DNA purity and amount.

For the rs1815739 SNP region containing the C/A-T missense mutation in the *ACTN3* gene, real-time PCR was used with particular primers. Probes were used to label the PCR products to be amplified. Melting curve analysis was used to distinguish between mutant and wild types. The analysis was carried out using specialized software. The results were confirmed using control DNA. Negative and positive controls were used to further validate the PCR process.

FMS

The FMS test consists of seven sub-tests (deep squat, hurdle step, in-line lunge, shoulder mobility, active

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straight-leg raise, trunk stability push-up and rotary stability). Prior to the measurements, information about the test was given by the applicators and the movements were demonstrated. In the test, each movement was repeated 3 times. The participants were asked to report any pain or unrest that may occur during the application of the movements to the expert making measurements. During scoring, the participants' scores from the right and left sides of their bodies were recorded. The lowest score achieved by a participant from a movement was considered as the result of the test. Each test is scored between 0 and 3 in itself. The maximum FMS test score is 21.[18,19] The test is scored as follows: 0 points if the participant feels pain during a movement; 1 point if the participant was unable to perform or complete a movement during the test; 2 points if the basic form of a movement was demonstrated but completed with some compensation; and 3 points if the basic standard of a movement was demonstrated accurately without compensation.

DATA ANALYSIS

The statistical analysis of the data was performed using the SPSS 25.0 program. In the evaluation of the data, descriptive statistical methods (number, percentage, mean and standard deviation) were used. Before performing any analysis on the data, the study determined whether they met the requirements for parametric tests. To that end, the variables were tested for normality while Kolmogorov-Smirnov and Levene's tests were used for homogeneity of variance. As result of these tests, non-parametric tests were performed for the variables distributed. Therefore, Mann-Whitney U test (by comparing the variables of 2 genotypes) was performed to examine the genotypic differences among the female runners, while the Kruskal-Wallis test was used for the male and overall genotypes. In order to determine which groups caused the significant difference determined after Kruskal-Wallis, the Mann-Whitney U test technique was used. The hypotheses were tested with a confidence interval of 95% and a significance level of $p<0.05$.

RESULTS

The present study aims to clarify whether there are any associations between FMS and the *ACTN3* (rs1815739) polymorphism. Three groups that were male runners, female runners and total runners have been chosen to asses this aim. Our results underlined that there were not any siginificant diffirences for association of the rs1815739 polymorphism with FMS within the total runners ($p>0.005$; Table 3). Importantly, the association of the rs1815739 polymorphism with female and male runners were statistically significant ($p=0.033$, $p=0.031$; Table 1, Table 2 respectively).

TABLE 1: rs1815739 association with the FMS within the female runners.

Gender	Variable	Genotype	n	$\bar{X}$	Total ranking	**Mann-Whitney U	Z	p value
Female	FMS	C/C	8	11.00	75.50			
		C/T	8	6.00	134.50	12.000	-2.132	**0.033***
		T/T	-	-	-			

***p<0.05;** **Mann-Whitney U test (by comparing the variables of 2 genotypes); FMS: Functional movement screen.

TABLE 2: rs1815739 association with the FMS within the male runners.

Gender	Variable	Genotype	n	Average ranking	df	**X^2	p value	Significant difference
Male	FMS	C/C[A]	10	11.70				B>A
		C/T[B]	10	20.60	2	6.977	**0.031***	B>C
		T/T[C]	9	12.44				

***p<0.05;** Kruskal-Wallis (by comparing the variables of 3 genotypes); FMS: Functional movement screen.

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TABLE 3: rs1815739 association with the FMS within the total runners.

Gender	Variable	Genotype	n	Average ranking	df	**X^2	p value	Significant difference
Total	FMS	C/C[A]	18	23.94				
		C/T[B]	18	25.42	2	3.171	0.205	--
		T/T[C]	9	16.28				

* **p<0.05;** **Kruskal-Wallis (by comparing the variables of 3 genotypes); FMS: Functional movement screen.

DISCUSSION

When studies conducted in the field of sports genetics are examined, it is observed that despite certain improvements in injury awareness and preventive strategies, the incidence of these injuries is still high. Thus, in the present study, the *ACTN3* gene polymorphism and FMS values of licensed runners competing at the national level in different clubs affiliated to the Turkish Athletic Federation were evaluated. According to the findings obtained, in the FMS assessment of the female runners, it was determined that the runners with the CC genotype had significantly higher mean score than those with the CT genotype while in the male runners, the runners with the CT genotype had a significantly higher mean score than those with the TT genotype. However, in the FMS evaluation of the overall runners, no significant difference was found based on genotype. While we expected to see significantly TT genotype lower FMS score for female, male overall runners, our findings did supports only female and male claim.

When the literature related to the *ACTN3* gene polymorphism is reviewed, many studies on non-impact injuries are found. For example, in the study conducted by Moreno et al. on marathon runners, it was reported that although athletes with the XX genotype were more prone to injury compared to athletes with the RX and RR genotypes, they also had a higher incidence rate of muscle injuries compared to the athletes with the RX and RR genotypes. In addition to these, it was determined that runners with the XX genotypes were 2 times more likely to be exposed to sudden onset muscle injury compared to runners with the RR genotype.[21] In another study on marathon runners, it was determined that athletes with the XX genotype experienced more severe lower extremity muscle pain compared to athletes with the RR genotype.[22] In a study conducted on professional football players, it was determined that footballers with the XX genotype were more susceptible to muscle damage following eccentric exercise compared to footballers with the RX and RR genotypes.[23] In the study conducted by Kim et al. with Korean elite ballet dancers, it was reported that the incidence of ankle injury was 4.65 times higher in ballet dancers with the XX genotype compared to those with the RX and RR genotypes.[24] Similar studies supporting these findings are also found.[25,26] In the related literature, it was reported that the RR genotype had a protective effect on injury. Additionally, the fact that the XX genotype is more common in injured individuals indicates that it may be a risk factor for non-impact injury.[33] Based on the fact that the number of individuals with the RR genotype is higher in the present study, it can be said that this is consistent with the literature and that these individuals carry a lower risk of suffering non-impact injuries. There are also different studies.[3,32] Different results could be a result of comparison of the athletes from diverse disciplines, type of training, studying with non-homogenous and limited groups, and limited history of the participants such as unclear ethnicity, sport experiences, and epigenetics.

The functions of FMS, which also has an important place in pre-season tests, are commonly utilized to reduce risk factors that may cause injuries, prevent the recurrence of injuries and determine the risk of injury. When previous studies on FMS are examined, in the study conducted by Bulğay et al. with long-distance runners, although no significant difference was found between the runners with and without

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a history of injury, the athletes with no history of injury scored higher.[27] In another study, it was reported that athletes with a history of injury scored higher compared to athletes without a history of injury, although this difference was not significant.[28] While there are similar studies in the related literature, there are also differing studies. In studies with different features, it was reported that athletes with a history of injury may perform better than those without a history of injury as a result of specialized injury treatment, such as physical therapy or strength training.[27] It can be said that conducting genetic tests in addition to field and laboratory tests in order to minimize the non-impact injury levels of runners may contribute to a healthier interpretation of injuries in runners.

With the movement patterns included in the FMS test battery, potential injuries can be prevented and athletic performance can be increased.[29] In order to determine FMS scores as a result of core training, the core stability training program conducted 3 days a week for 8 weeks was found to result in a significant improvement in the overall scores of the athletes, particularly in the hurdle step movement pattern. As a result, it was reported to increase FMS scores and dynamic postural control.[30] In another study, Bodden et al. applied an eight-week individual exercise program to professional martial arts athletes 4 days a week and reported an increase in FMS scores.[31]

While the fact that the group of runners in the present study are homogeneous is regarded as the strong aspect of the study, the number of runners that could be reached limits the study. It is thought that findings obtained from future studies conducted with different sports branches and wider sample groups will provide significant contributions to sport sciences field experts and practitioners. Another limitation of the study was the menstrual cycle not included in the calculation when measurements were taken from female participants.

CONCLUSION

Based on the findings obtained and the literature review performed, it can be said that XX genotype carriage may be a risk factor for injury, and that RR genotype carriage has a protective effect in non-impact injuries. It can also be said that runners with high mean scores from the FMS test battery carry a lower risk of injury, and that this may be an effective factor in performance improvement.

It is thought that the FMS test battery and the *ACTN3* gene polymorphism, which is responsible for and among the significant variables of non-impact injuries, may be effective in predicting injuries beforehand, reducing risk factors that may cause injury, the level of muscle damage and the type of muscle injury. Based on this, it can be suggested that genetic tests are taken into consideration as well as field and laboratory tests in order to minimize injuries in runners, and that these approaches are developed as much as possible. More replication studies are needed to provide clearer information about the research.

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: *Celal Bulgay;* ***Design:*** *Celal Bulgay, Meriç Ödemiş, Damla Selin Yıldırım;* ***Control/Supervision:*** *Işık Bayraktar, Korkut Ulucan, Mesut Cerit;* ***Data Collection and/or Processing:*** *Mehmet Ali Ergün, Celal Bulgay, Korkut Ulucan;* ***Analysis and/or Interpretation:*** *Celal Bulgay, Işık Bayraktar;* ***Literature Review:*** *Celal Bulgay, Damla Selin Yıldırım;* ***Writing the Article:*** *Celal Bulgay, Işık Bayraktar, Damla Selin Yıldırım;* ***Critical Review:*** *Korkut Ulucan, Mesut Cerit, Mehmet Ali Ergün;* ***References and Fundings:*** *Işık Bayraktar, Mehmet Ali Ergün;* ***Materials:****Celal Bulgay, Işık Bayraktar, Mehmet Ali Ergün.*

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