Eurasian Research in Sport Science • Avrasya Spor Bilimleri Araştırmaları • ERISS • Volume/Cilt 9 • Issue/Sayı 2 • December/Aralık 2024, ISSN 2636-8269, ss. 64-71 • DOI: 10.29228/ERISS.46

ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

Alpha-Actinin-3 (*ACTN3*) rs1815739 Polymorphism in HADO Athletes: A Pilot Study

HADO Sporcularında Alfa-Aktin-3 (ACTN3) rs1815739 Polimorfizmi: Bir Pilot Çalışma

Beste TACAL ASLAN*
Dudu Banu ÇAKAR**
Özlem Özge YILMAZ***
Tolga POLAT****
Rıdvan EKMEKÇİ****
Korkut ULUCAN*****

Abstract

There is growing interest in studying the genetic profile of high-performing athletes and how these genetic factors impact their athletic abilities. *ACTN3* is a highly influential gene in determining athletic capabilities. This study aims to examine the *ACTN3* rs1815739 genetic variant in HADO athletes. Eight HADO athletes and 30 individuals as a control participated in this study. After isolating DNA from buccal epithelial cells, genotyping was performed using Real-Time PCR. Genotype distribution among athletes was 4 (50%) CC, 2 (25%) CT, and 2 (25%) TT. In the control group, genotypes were 15 (50%) CC, 8 (26.7%) CT, and 7 (23.3%) TT. There was no significant difference between groups (p=0.9930). Allele distribution in athletes was 10 (62.5%) C and 6 (37.5%) T, while the control group had 38 (63.3%) C and 22 (36.7%) T alleles. The statistical comparison showed no significant difference (p=0.9510). The results showed that the CC genotype was higher compared to the CT and TT genotypes. At the same time, the C allele was also found to be higher

* Asst. Prof., Marmara University, Faculty of Dentistry, Department of Basic Medical Sciences, Istanbul, Türkiye, btacal@gmail.com, ORCID: 0000-0001-5271-7917

** Asst. Prof., Istanbul Aydın University, Faculty of Fine Arts, Digital Game Design, Istanbul, Türkiye, dbcakar@gmail.com, ORCID: 0000-0002-0092-3062

*** Res. Asst., Marmara University, Faculty of Dentistry, Department of Basic Medical Sciences, Istanbul, Türkiye, ozlem.ozge@marmara.edu.tr, ORCID: 0000-0002-4085-6159

**** Res. Asst., Marmara University, Faculty of Dentistry, Department of Basic Medical Sciences, Istanbul, Türkiye, tolga.polat@marmara.edu.tr, ORCID: 0000-0002-2064-6613

***** Prof. Dr., Pamukkale University, Faculty of Sports Sciences, Department of Sports Management, Denizli, Türkiye, rekmekci@pau.edu.tr,ORCID: 0000-0001-5085-5988

******Prof. Dr., Marmara University, Faculty of Dentistry, Department of Basic Medical Sciences, Istanbul, Türkiye, korkutulucan@hotmail.com ORCID: 0000-0002-1304-9386

How to cite this article/Atıf için: Aslan Tacal, B., Çakar, D.B., Yılmaz, Ö.Ö., Polat, T., Ekmekçi, R., Ulucan, K. (2024). Alpha-Actinin-3 (ACTN3) rs1815739 Polymorphism in HADO Athletes: A Pilot Study. *Eurasian Research in Sport Science, 9*(2), 64-71. DOI: 10.29228/ERISS.46

Makale Gönderim Tarihi: 14.06.2024 **Yayına Kabul Tarihi:** 19.09.2024

in percentage than the T allele. Our research has shown that the *ACTN3* rs1815739 polimorphism C allele associated with sprinting and strength sports may confer an advantage in HADO. This is the first study of athletic performance in HADO athletes.
Keywords: *ACTN3*, HADO, Polymorphism, Sport genetics

Öz

Yüksek performanslı sporcuların genetik profillerinin incelenmesine ve bu genetik faktörlerin atletik yeteneklerini nasıl etkilediğine yönelik ilgi artmaktadır. *ACTN3*, atletik yetenekleri belirlemede oldukça etkili bir gendir. *ACTN3* rs1815739 varyantı 11. kromozomda (11q13.1) bulunur ve ekson 16'da sitozinden timine geçiş nedeniyle oluşur. Bu çalışma, HADO sporcularındaki *ACTN3* rs1815739 genetik varyantını incelemeyi amaçlamaktadır. Bu çalışmada sekiz HADO sporcusu yer aldı. Bukkal epitel hücrelerinden DNA izole edildikten sonra, Real-Time PCR (PCR) kullanılarak genotipleme yapıldı. Sporcular arasındaki genotip dağılımı 4 (%50) CC, 2 (%25) CT ve 2 (%25) TT idi. Kontrol grubunda genotipler 15 (%50) CC, 8 (%26,7) CT ve 7 (%23,3) TT idi. Gruplar arasında anlamlı bir fark yoktu (p=0.9930). Sporculardaki alel dağılımı 10 (%62,5) C ve 6 (%37,5) T iken, kontrol grubunda 38 (%63,3) C ve 22 (%36,7) T aleli vardı. İstatistiksel karşılaştırma anlamlı bir fark göstermedi (p=0,9510). Sonuçlar CC genotipinin CT ve TT genotiplerine kıyasla daha yüksek olduğunu gösterdi. Aynı zamanda C aleli de T alelinden yüzde daha yüksek bulundu. Araştırmamız, sprint ve kuvvet sporlarıyla ilişkili *ACTN3* rs1815739 polimorfizmi C alelinin HADO sporunda avantaj sağlayabileceğini bildirmiştir. Bu çalışma, HADO sporcularında atletik performans üzerine yapılan ilk çalışmadır.
Anahtar Kelimeler: *ACTN3*, HADO, Polimorfizm, Spor genetiği

INTRODUCTION

The Human Genome Project has enabled the determination of the structure, functions and effects of our genes, which are estimated to be around 20-25 thousand, on our biological systems. At the beginning of these biological systems, there are physiological, biochemical and psychological factors that affect athletic performance (Çorak et al., 2017). Athletic performance is often a mix of genetic profile and external influences unique to each person and is thought to be the result of various factors like diet, mental well-being, guidance, and natural abilities (Akçamlı et al., 2018; Ulucan et al., 2014).

Regular training enhances athletic performance by influencing genetic factors which have varying impacts on an individual's performance. The effect of genetic and mental factors on the creation and development of athletic performance in athletes with regular training has been demonstrated by studies carried out to date (Ulucan, 2016). In the process of building and improving athletic abilities, it is crucial for muscle metabolism to be functioning at its peak level. Genetic differences play a significant role in metabolism functions, impact activities such as physical exercise at the cellular level. Hence, it is expected that athletes possess the most advantageous genetic traits in their genotypes (Polat et al., 2020).

HADO was created as an AR sport by Hiroshi Fukuda in 2014 and has grown in popularity since then. Since 2014, HADO has been a popular sport in over 26 countries, incorporating technology into gameplay. HADO has three primary characteristics. HADO is a physical game that demands exceptional footwork, quickness, and agility. It is crucial for players to move quickly and change direction suddenly. Therefore, training includes sprint work, plyometric exercises, and agility-requiring track work. Explosive power training is essential, particularly for the lower and upper body muscles, to enable

quick reactions and fast energy ball throws. Additionally, cardiovascular endurance training is applied to ensure high performance throughout the game. Also, mental focus training is conducted to make fast and accurate decisions during the HADO game. This is supported by meditation, visualization, and stress management techniques. In HADO, players compete not only against their opponents but also against a clock that counts down from 80 seconds. The sport combines physical ability with strategic thinking, allowing players to excel in either athleticism or tactics. HADO is a game of attack and defense, where players determine their skill sets before stepping onto the field. The depth of strategy can engage teams with greater knowledge of the game more deeply into the sport (Araki et al., 2017).

In sports genetics, most of the studies on athletic performance involve the alpha-actinin-3 (*ACTN3*) gene. In mammals, actin protein exists in four different forms; ACTN1, ACTN2, ACTN3, and ACTN4. The ACTN1 protein plays a role in cellular functions by connecting actin filaments in cells. The ACTN2 protein binds to the Z-line of actin filaments running anti-parallel at sarcomeres in muscle tissue. Although the ACTN4 protein has structurally similar properties to the ACTN1 protein, this protein is known as the non-muscle isorform and has different properties (Murphy and Young, 2015; Otey and Carpen, 2004).

The ACTN3 protein plays an important role in muscle contraction by interacting with actin fibers and transmitting signals within cells. The ACTN3 protein, found exclusively in skeletal muscle and linked to athletic performance, plays a key role in quick muscle contractions in glycolytic type and type-II X muscle fibers (Seto et al., 2013). The *ACTN3* gene, found on chromosome 11q13.1, contains the instructions for producing the ACTN3 protein. The ACTN3 protein is found in the Z lines of sarcomeres in skeletal muscle where it helps bind actin fibrils during muscle contractions. The *ACTN3* rs1815739 polymorphism, characterized by a substitution of cytosine (C) with thymine (T) in the 16th exon of the *ACTN3* gene, is a widely researched area in the realm of sports genetics. This alteration leads to a single substitution in a base, leading to the creation of a stop codon (X) instead of the codon (R) that normally codes for arginine. People with this genetic variation have a lack of ACTN3 (Kikuchi and Nakazato, 2015; Eroğlu et al., 2018). In sports genetics studies on *ACTN3* rs1815739 polymorphism, CC genotype was associated with enhanced sprint/power ability, and TT genotype was associated with endurance ability (Ulucan et al., 2013).

The aim of this study is to investigate the distribution of the *ACTN3* rs1800795 polymorphism in sports requiring fast reflexes, agility and explosive power, such as HADO, and to investigate whether individuals carrying the C allele may have an advantage in this sport, while individuals carrying the T allele may have different advantages in situations requiring strategy and endurance.

METHODS

Study Group

Since our study is the first to be conducted on HADO sports in Turkey, a power analysis could not be performed due to the small number of athletes. Eight HADO athletes voluntarily participated in our study. As a control group, 30 individuals who did not exercise regularly participated in our study.

The study protocol was approved by the Üsküdar University Ethics Committee (61351342/020-31) and was prepared in accordance with the guidelines of the Helsinki Declaration-2 (2015). Before the study, all participants signed consent forms containing all the information such as the study protocol, results, and evaluation of the results.

Genotyping

DNA isolations from the buccal cells of the athletes participating in the study were performed with a commercially obtained PureLink DNA isolation kit (Invitrogen, Thermo Fisher Scientific, Inc.). Genotyping of the *ACTN3* rs1815739 polymorphism was performed using Real-Time PCR on a StepOnePlus (Thermo Fisher Scientific, Inc.) and by using Taqman SNP Genotyping Assays genotyping kits according to the manufacturers' protocols (cat. no. 4362691, Thermo Fisher Scientific, Inc.). C and T alleles were determined using FAM and VIC primers, respectively (Fig. 1). For a total volume of 10 µl reaction, 5 µl of Genotyping Master Mix (Applied Biosystems, Foster City, CA), 3.5 µl of nuclease-free H_2O (Thermofisher, USA), 0.5 µl of genotyping test (Applied Biosystems), and 1 µl of DNA were used. The sequences of the TaqMan Probe used for genotyping are listed in Figure 2.

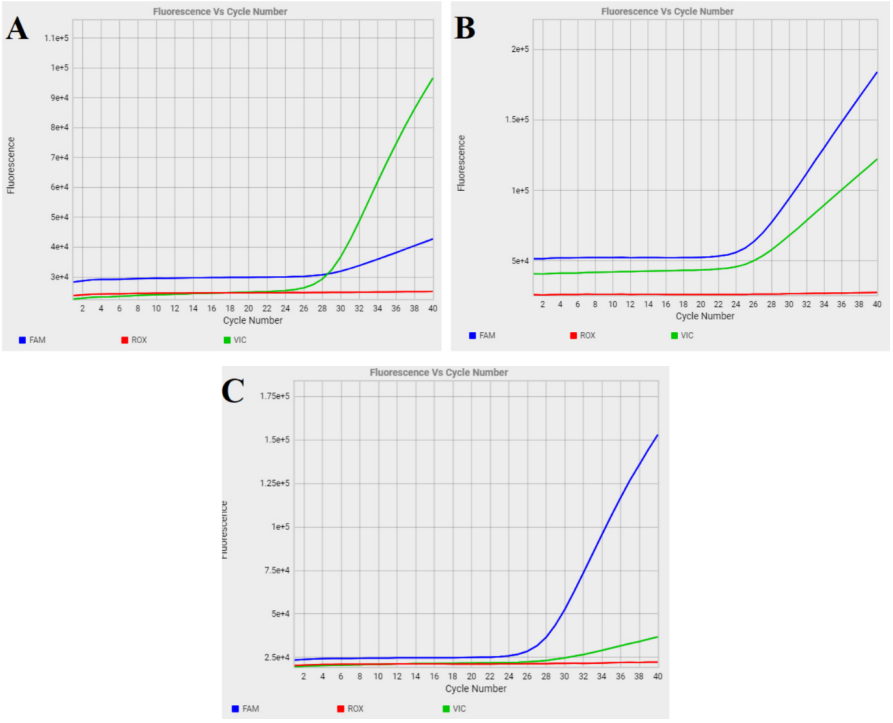

Figure 1: Quantitative PCR amplification of the CC, CT, and TT genotypes of *ACTN3* rs1815739 polymorphism. FAM indicates the T (X) allele (blue curve), whereas VIC (green curve) indicates the C (R) allele. (A) The single blue curve indicates the homozygous genotype of CC (RR) whereas (B) the blue and green curves indicate the heterozygous genotype of CT (RX) and the single green curve indicates the homozygous genotype of TT (XX).

Statistical Analysis

Genotype distribution and allele frequencies between groups of athletes and controls were then compared by $\chi 2$ testing using the SPSS (version 25.0 for Windows, SPSS, Chicago, IL, USA) program in the statistical analysis of the obtained results. $p<0.05$ value was accepted as statistically significant.

Table 1. Sequences of the TaqMan probe used for genotyping *ACTN3* rs1815739 polymorphism.

qPCR	**Sequence, 5'-3'**
FAM/VIC	CAAGGCAACACTGCCCGAGGCTGAC[**T/C**]GAGAGCGAGGTGCCATCATGGGCAT

RESULTS

Among the HADO athletes, 4 (50%) had the CC genotype, 2 (25%) had the CT genotype, and 2 (25%) had the TT genotype. When examining the distribution of alleles, 10 (62.5%) C alleles and 6 (37.5%) T alleles were counted. In the control group (n = 30), 15 had the CC genotype, 8 had the CT genotype, and 7 had the TT genotype. 38 (63.3%) C alleles and 22 (36.7%) T alleles were counted. The distribution of genotypes and alleles among the athletes is summarized in Table 1.

Table 1: Genotype and allele distribution of *ACTN3* rs1815739 in surveyed HADO players.

	Genotype			p Value	Allelic Distribution		p Value
	CC	**CT**	**TT**		**C**	**T**	
Athlete (8)	4	2	2	0.9930	10	6	0.9510
Percentage	50%	25%	25%		62.5%	37.5%	
Control (30)	15	8	7		38	22	
Percentage	50%	26.7%	23.3%		63.3%	36.7%	

Significance was assessed at least at the $p<0.05$ level. Comparisons with the control group were performed using chi-square tests.

DISCUSSION AND CONCLUSION

Research has indicated that athletic performance is influenced by a combination of environmental factors and various genetic groups. Athletic performance is influenced by a variety of factors, including the combination of environmental elements and various genetic groups (Ulucan, 2016). It is stated in scientific studies that the most important factor affecting athletic performance is genetic differences. Genetic analyzes are performed to obtain the desired results and to find the optimal performance of the athletes. Single nucleotide polymorphism studies enable the identification of candidate genes related to athletic performance and the increasing number of candidate genes day by day (Macarthur and North, 2005).

The *ACTN3* rs1815739 polymorphism has proven to be associated with elite strength performance in many ethnic populations. Egorova et al. (2014) reported that CC genotype (46.3%) and C allele (88.5%) were found to be percent higher in their study with 240 Russian football players. Mutlucan et al. (2017), reported that the CC genotype (47.5%) and the C allele (66.3%) were higher in percentages

in a study involving 40 professional football players. Eroğlu et al. (2018), in a study involving 23 national athletes, 27 amateur athletes, and 34 sedentary individuals, it was found that the CT genotype was more prevalent. Upon analyzing the allelic distributions, it was discovered that national athletes had a higher prevalence of the T allele, whereas amateur athletes and sedentary individuals had a higher prevalence of the C allele. Kikuchi et al. (2016) divided the athletes into two as strength and endurance in their study on 1057 Japanese athletes. It has been reported that CT genotype (51%) and C allele (51.2%) are higher in strength athletes, and CT genotype (49%) and T allele (52.8%) in endurance athletes. According to Polat et al. (2020) reported that the CT genotype (54.5%) and the C allele (72.7%) were higher in percentages in a study involving 11 Turkish bodybuilders for the *ACTN3* rs1815739 polymorphism. Yang et al. (2021), in their study of 125 Chinese elite athletes, divided the athletes into two as strength and endurance. *ACTN3* rs1815739 polymorphism CC genotype (50.7%) and C allele (68.5%) were found to be percent higher in strength athletes. In endurance athletes, the CT genotype (46.2%) was found to be percent higher. Pranckeviciene et al. (2021) reported that the CT genotype (56.7%) was percent higher than the CC and TT genotypes in a study they conducted on 180 elite athletes from Lithuanians. In addition, it was reported that the C allele (59.4%) was percent higher than the T allele. Söyler et al. (2024) show that there is no statistically significant difference in *ACTN3* gene polymorphism between professional and amateur football players, but professional football players are superior in anaerobic performance parameters such as 10m sprint, 30m sprint and anaerobic power. In genotype analyses, CT genotype C allele was found to be more common in professional and amateur football players.

In our study, we determined the distribution of *ACTN3* rs1815739 polymorphism in HADO athletes. The CC genotype (50%) was higher for the *ACTN3* genotype compared to the CT and TT genotypes. When the allele distributions were examined, the C allele (62.5%), which is considered as wild type allele, was found to be higher in percentage than the T allele. The C allele is linked to quick sarcomere contraction and is viewed as the dominant allele. The TT genotype tends to have a lower percentage of fast-twitch muscle fibers and lower power performance (Pickering et al., 2019). It is believed that individuals with a CC genotype have greater muscle strength and power, and this genotype is more prevalent among sprint and power athletes than in the general population. Our research reported that the C allele of the *ACTN3* rs1815739 polymorphism may provide superiority in HADO as well as in sprint and strength sports. Even so, the *ACTN3* rs1815739 polymorphism, the results could not be compared as no data were found regarding HADO athletes.

The limitation of our study is the small sample size, as we only considered the polymorphism results of the athletes who applied the same training program as an independent variable. We think that this study on HADO national team athletes will both contribute to the literature and lead the studies to be done in the future thanks to the findings obtained.

Acknowledgement: We would like to thank the founder of HADO Türkiye, Koray Erdemir and Can Duraslan.

Conflicts of Interest: There is no personal or financial conflict of interest within the scope of the study.

Authors' contributions: Design of the study: BTA %20, DBÇ %30, RE %25, KU %25 contributed. Methodology: BTA %30, ÖÖY %35, TU %35 contributed. Statistical analysis: ÖÖY %50, TP %50 contributed. Manuscript writing: BTA %50, ÖÖY %25, TP %25 contributed. Review and editing: DBÇ %20, RE %40, KU %40 contributed.

Ethics Committee: Üsküdar University Non-Interventional Ethics Committee (61351342/020-31). Date: 30.04.2024

REFERENCES

Akçamlı, D., Sipahi, S., Yüksel, İ., Kavas, N. C., Polat, T., Sercan, C., Kapıcı, S., Eken, B. F., & Ulucan, K. (2018). Futbolcularda Peroksizom Proliferatör – Aktive Reseptör Alfa rs4253778 Polimorfizm Dağılımının Belirlenmesi. *Eurasian Research in Sport Science*, *3*(2), 75-79. https://doi.org/10.22396/ERISS.2018.37

Araki, H., Fukuda, H., Motoki, T., Takeuchi, T., Ohta, N., Adachi, R., ... & Kakeya, N. (2017). "HADO" as Techno Sports was born by the fusion of IT technology and sports. *ReVo*, 36-40.

Çorak, A., Kapıcı, S., Sercan, C., & Ulucan, K. (2017). A pilot study for determination of anxiety related SLC6A4 promoter "S" and "L" alleles in healthy Turkish athletes. *Cellular and Molecular Biology, 63*(5), 29-31. https://doi.org/10.14715/cmb/2017.63.5.6

Egorova, E. S., Borisova, A. V., Mustafina, L. J., Arkhipova, A. A., Gabbasov, R. T., Druzhevskaya, A. M., Astratenkova, I. V., & Ahmetov, I. I. (2014). The polygenic profile of Russian football players. *J Sports Sci.*, *32(13)*, 1286–1293. https://doi.org/10.1080/02640.414.2014.898853

Eroğlu, O., Zileli, R., Nalbant, M. A., & Ulucan, K. (2018). Prevalence of alpha actinin-3 gene (ACTN3) R577X and angiotensin converting enzyme (ACE) insertion / deletion gene polymorphisms in national and amateur Turkish athletes. *Cellular and Molecular Biology*, *64*(5), 24–28. https://doi.org/10.14715/cmb/2018.64.5.4

Kikuchi, N., & Nakazato, K. (2015). Effective utilization of genetic information for athletes and coaches: focus on ACTN3 R577X polymorphism. *Journal of exercise nutrition & biochemistry*, *19*(3), 157-164. https://doi.org/10.5717/jenb.2015.150.93001

Kikuchi, N., Miyamoto-Mikami, E., Murakami, H., Nakamura, T., Min, S.K., Mizuno, M., Naito, H., Miyachi, M., Nakazato, K., & Fuku, N. (2016) ACTN3 R577X genotype and athletic performance in a large cohort of Japanese athletes. *European Journal of Sport Science*, *16*(6), 694-701. https://doi.org/10.1080/17461.391.2015.1071879

MacArthur, D. G., & North, K. N. (2005). Genes and human elite athletic performance. *Human genetics*, *116*(5), 331-339. https://doi.org/10.1007/s00439.005.1261-8

Murphy, A. C., & Young, P. W. (2015). The actinin family of actin cross-linking proteins – a genetic perspective. *Cell & Bioscience*, *5*, 49. https://doi.org/10.1186/s13578.015.0029-7

Mutlucan, H., Bıyıklı, T., Eken, B. F., Sercan, C., Kapıcı, S., & Ulucan, K. (2017). Türk Profesyonel Futbolcularda Alfa-Aktinin-3N R577 X Polimorfizminin İncelenmesi. *Marmara Üniversitesi Spor Bilimleri Dergisi*, *2*(2), 1-7. https://doi.org/10.22396/sbd.2017.26

Otey, C. A., & Carpen, O. (2004). Alpha-actinin revisited: a fresh look at an old player. *Cell Motil Cytoskeleton*, *58*(2), 104-11. https://doi.org/10.1002/cm.20007

Pickering, C., Kiely, J., Grgic, J., Lucia, A., & Del Coso, J. (2019). Can Genetic Testing Identify Talent for Sport? *Genes*, *10*(12), 972. https://doi.org/10.3390/genes10120972

Polat, T., Dogan, C. S., Dogan, M., Akçay, T., & Ulucan, K. (2020). Distribution of αactinin3 rs1815739 and angiotensin1 converting enzyme InDel polymorphisms in Turkish bodybuilders. *Biomedical Reports*, *13*(6), 67. https://doi.org/10.3892/br.2020.1374

Pranckeviciene, E., Gineviciene, V., Jakaitiene, A., Januska, L., & Utkus, A. (2021). Total Genotype Score Modelling of Polygenic Endurance-Power Profiles in Lithuanian Elite Athletes. *Genes*, *12*(7):1067. https://doi.org/10.3390/genes12071067

Seto, J. T., Quinlan, K. G., Lek, M., Zheng, X. F., Garton, F., MacArthur, D. G., Hogarth, M.W., Houweling, P. J., Gregorevic, P., Turner, N., Cooney, G. J., Yang, N., & North, K. N. (2013). ACTN3 genotype influences muscle performance through the regulation of calcineurin signaling. *J Clin Invest*, *123*(10), 4255-63. https://doi.org/10.1172/JCI67691

Söyler, M., Zileli, R., Özkamçı, H., Diker, G., Sever, M. O., Bayrakdaroglu, S., ... & Yılmaz, Ö. Ö. (2024). Sprint and Anaerobic Power with the Soccer-Specific ACTN3 Gene: A Distintive Example. *Int. J. Morphol*, *42*(2), 538-548.

Ulucan, K. (2016). Literature Review of Turkish Sportsmen in Terms of ACTN3 R577X Polymorphism. *Clinical and Experimental Health Sciences*, *6*(1): 44–47. https://doi.org/10.5152/clinexphealthsci.2016.059

Ulucan, K., Bayyurt, G. M., Konuk, M., & Güney, A. **İ**. (2014). Effect of alpha-actinin-3 gene on Turkish trained and untrained middle school children's sprinting performance: a pilot study. *Biological Rhythm Research*, *45*(4), 509-514. https://doi.org/10.1080/09291.016.2013.867628

Ulucan, K., Göle, S., Altindas, N., & Güney, A. (2013). Preliminary Findings of α – Actinin-3 Gene Distribution in Elite Turkish Wind Surfers. Balkan journal of medical genetics, 16(1), 69–72. https://doi.org/10.2478/bjmg-2013-0020

Yang, R., Jin, F., Wang, L., Shen, X., Guo, Q., Song, H., Hu, J., Zhao, Q., Wan, J., & Cai, M. (2021). Prediction and Identification of Power Performance Using Polygenic Models of Three Single-Nucleotide Polymorphisms in Chinese Elite Athletes. *Frontiers in genetics*, *12*(726552), 1858. https://doi.org/10.3389/fgene.2021.726552