Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Original Research

Prevalence of alpha actinin-3 gene (*ACTN3*) R577X and angiotensin converting enzyme (*ACE*) insertion / deletion gene polymorphisms in national and amateur Turkish athletes

Onur Eroğlu[1,2], Raif Zileli[2,3], M. Ali Nalbant[1], Korkut Ulucan[4,5*]

[1] Department of Molecular Biology and Genetics, Faculty of Art and Science, Bilecik Seyh Edebali University, Bilecik, Turkey
[2] Biotechnology Research and Development Center, Bilecik Seyh Edebali University, Bilecik, Turkey
[3] Vocational School of Health, Bilecik Seyh Edebali University, Bilecik, Turkey.
[4] Department of Medical Biology and Genetics, Marmara University, Faculty of Dentistry, Istanbul, Turkey
[5] Department of Molecular Biology and Genetics, Üsküdar University, Faculty of Engineering and Natural Sciences, Istanbul, Turkey

Correspondence to: korkutulucan@hotmail.com

Received November 6, 2017; **Accepted** April 15, 2018; **Published** April 30, 2018

Doi: http://dx.doi.org/10.14715/cmb/2018.64.5.4

Abstract: Studies to date showed the importance of alpha- actinin-3 (*ACTN3*) R577X and angiotensin converting enzyme (*ACE*) ID polymorphisms on determining athletic performance. Therefore, in this study, we aimed to examine polymorphisms given to Turkish athletes and compare them with sedentary individuals. Genomic DNAs were extracted from peripheral blood by using commercially available DNA isolation kit (Macherey-Nagel, NucleoSpin®, Germany). For this study, a total of 84 volunteers (23 national athletes, 27 amateur athletes and 34 sedentary controls) was recruited. *ACE* ID genotypes were determined by conventional polymerase chain reaction, and *ACTN3* R577X polymorphisms by polymerase chain reaction- restriction fragment length polymorphism methodology. In *ACTN3* R577X polymorphism, RX was the dominating genotype, and we detected no RR genotype in national athletes. (no RR genotype was detected in national athletes) X allele is more frequent in national athletes and R allele was more frequent in both amateur athletes and control group. II genotype was more frequent in national athletes and in control group, whereas DD genotype was more frequent in amateur athletes for *ACE* ID polymorphism. When we consider alleles, D allele was found more frequently in amateur athletes and control group whereas I allele was more frequent in national athletes in *ACE* ID polymorphism. For ACTN3, X allele was superior to R allele. *ACTN3* R577X and *ACE* ID polymorphisms were important biomarkers in determining athletic performance. However, our results in Turkish athletes suggest that ACE D allele and ACTN3 X alleles may be beneficial to athletes potentially, regardless of the distance they perform.

Key words: Sports; Genetics; Athletic performance; Athlete.

Introduction

It has been known for a long time that athletic performance can develop in accordance with intense exercise programs (1). However, it seems that some individuals are inherently athletic. The average performance of individuals with innate abilities is perfect, even before training and after training. The athletic potential is predetermined by inherited traits, and the prediction of the degree of training response before starting training program causes many controversies. This feature is most likely due to the strong association between genetic and environmental factors contributing to sporting performance. Whether there is a limit for the athletic performance and the sporting activities or not is still a question that remains a mystery. That is, athletic ability consists of many physiological and psychological interactions determined by many factors (2). In the same way, sportive performance is related with the success of a sporty activity. Sports genetics is relatively a new concept that examines the genetic factors which influence the improvement and development of athletic performance.

Alpha- actinin-3 (*ACTN3*) protein is one of the important proteins having functions in muscle contraction, formation of Z- lines of sarcomeres and signal transduction. This protein is coded by ACTN3 gene and considered to be one of the first structural gene that associates with athletic performance (3). It is one of the members of a four-member gene family, *ACTN1-4* (4). *ACTN3* is the gene responsible for coding alpha -actinin- 3 in humans and the genes' expression is restricted to type-2 fast twitch fibres. North et al. (1999) reported a variation in the gene, resulting a premature protein because of a stop codon at position 577 (C>T transition) which alters the designation for the amino acid arginine to a stop codon (R577X; dbSNP rs1815739) in exon 16. Recent studies associated with the 577R allele and RR genotype with top-level, power-orientated athletic performance (3). *ACTN3* R577X polymorphism has been reported to be associated with elite athletic status (3), endurance athletes (6,7) and many other groups (8,9).

The angiotensin converting enzyme (ACE) gene is responsible for encoding ACE enzyme, and is located at 17q23, comprises 26 exons and 25 introns. It constitutes several polymorphisms, but in athletic performance evaluation, an insertion (I) or a deletion (D) of a 287 base pair (bp) within intron16 InDel polymorphisms found in ACE gene have importance.

Depending on the insertion and/or deletion status; 3 different genotypes may result as; insertion/insertion (II), insertion/deletion (ID), and deletion/deletion (DD). ACE InDel polymorphism is associated with circulating

and tissue ACE amounts. ACE enzyme is a key molecule in renin- angiotensin system and related with blood pressure. DD genotype is reported to have higher ACE concentrations when compared to ID and II genotypes (10). Many of the studies to date have suggested that success in speed- strength disciplines, like sprinting, short distance swimming, long jump and high jump are associated with *ACE* DD genotype (11). On the other hand, individuals with II genotype have a lower ACE serum concentration and have more success in endurance related disciplines like medium and long distance running, race walking and rowing (12).

There are many parameters that successful athletes have, some of biological attributes are related with lengths of legs and arms, body shape, muscle strength, muscle metabolism and mental attributes which coordinate the body. Genes play essential roles in determining these features. Previous studies in Turkish athletes are very limited, especially in terms of *ACE* ID and *ACTN3* R577X polymorphism. In this study, we aimed to determine the genotype and allele distribution of *ACTN3 R577X* and *ACE* ID polymorphisms in short and long distance runners.

Materials and Methods

Participants

A total of 84 individuals, 23 national athletes (n: 12, short distance, n: 6, medium distance, n: 5, long distance), 27 amateur athletes (n: 10, short distance, n: 6, medium distance, n: 11, long distance) and 34 sedentary individuals is enrolled in the study. Sedentary individuals are all healthy, 18- year-old volunteers having education in Bilecik Seyh Edebali University, and perform a physical activity 2 days a week and less than 15 minutes a day. Before taking blood from all the individuals, the participants were informed about the risks and benefits of this work and a written consent was obtained on their voluntary consent forms. Participants were informed about their results.

The study we conducted was approved by Eskişehir Osmangazi University Faculty of Medicine, Ethics Committee for Clinical Investigations (2016/22) and carried out in accordance with the Helsinki Declaration of the World Medical Association. This work was supported by Bilecik Seyh Edebali University Scientific Research Organization (2015-02.BSEÜ.13-01).

DNA isolation and genotyping

A commercially available DNA isolation (MACHEREY-NAGEL, NucleoSpin®, Germany) kit was used for DNA isolation. The purity of the isolated DNAs was performed on Nanodrop (Shimadzu Biotech), only the ones that have a A260 / A280 ratio range of 1.60-1.90 ng / ml and a concentration of 50-100 ng were included in the study.

ACTN3 genotyping

ACTN3 R577X polymorphism was detected by conventional polymerase chain restriction fragment length polymorphism (PCR- RFLP) by using the forward 5′-CTG TTG CCT GTG GTA AGT GGG-3′ and reverse 5′-TGG TCA CAG TAT GCA GGA GGG-3′, primers. PCR was performed by a denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 65°C for 30 sec and extension at 72°C for 30 sec, and a final extension for seven minutes at 72°C. Genotyping of the amplicons were maintained by restriction fragment length polymorphism methodology. *DdeI* (New England Biolabs) restriction enzyme was used to detect the genotypes and 3% agarose gel electrophoresis was used to analyse the fragments under UV light by ethidium bromide staining.

ACE genotyping

Conventional PCR amplification was used to genotype ACE InDel polymorphism. Primers 5'-CTGGAGACCACTCCCATCCTTTCT-3' and 5'- GATGTGGCCATCACATTCGTCAGT-3' were used for genotyping processes. PCR reactions were carried out in a 50 µL total volume containing 50- 100 ng genomic DNA, 1 mM of each primers, 50 mM KCl, 1 mM dNTP, 1.5 mM MgCl2, 10 mM Tris-HCl, pH 8.0, and 1.5 U Taq DNA polymerase enzyme. Initial denaturation at 94°C for 5 min, annealing at 58°C for 1 min, and extension at 72°C for 2 min were performed respectively. After the first amplification step, 30 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 2 min, were followed by a final elongation at 94°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 7 min were applied. Amplicons were separated by 1.5% agarose gel electrophoresis and visualized under ultraviolet light after standard ethidium bromide staining.

Allelic evaluation was performed by direct counting of the alleles.

Results

Our cohort consisted of 23 national, 27 amateur and 34 sedentary individuals. In our cohort, RX of *ACTN3* was the dominant genotype in three groups. Percentages

Table 1. *ACTN3* genotype and allelic distribution of athletes and sedentary participants in the study cohort.

	National Athletes (n= 23)				Amateur Athletes (n= 27)				Sedentary Individuals (n= 34)
	Short Distance (n= 12)	Middle Distance (n= 6)	Long Distance (n= 5)	Total	Short Distance (n= 10)	Middle Distance (n= 6)	Long Distance (n= 11)	Total	
RR	-	-	-	-	3	-	-	3	9
RX	9	4	4	17	7	6	9	22	17
XX	3	2	1	6	-	-	2	2	8
Alleles									
R	9	4	4	17	13	6	9	28	35
X	15	8	6	29	7	6	13	26	33

of RX genotype in national, amateur and sedentary participants were 74, 81 and 50 respectively. In national athletes, we detected no RR genotype and XX genotype were found in 26% of our national athletes. In amateur athletes, RR genotype was found in 11% of the athletes whereas 8% of the cases had XX genotype. RR and XX genotypes were found in 26% and 24% of sedentary participants. When we consider alleles, "R" allele was the dominant allele in both amateur and sedentary participants. The percentages of "R" allele were 37, 52 and 51 respectively in national, amateur and sedentary participants. 63% of the national athletes had X allele whereas 48% of the amateur and 49% of sedentary participants carried the same allele. *ACTN3* genotype and allele numbers of the athletes are given in Table 1.

According to the *ACE* results, *ACE* II genotype was superior in national athletes and in sedentary participants. In amateur athletes, DD genotypes was the dominant genotype. DD, ID and II frequencies were 35, 26 and 39 for national athletes; 41, 26, 33 for amateur athletes; 26, 21 and 53 for the sedentary participants respectively. "D" allele was found in 48% of national athletes, 54% of amateur athletes and 37% of sedentary individuals. "I" allele was more frequent in national athletes with a frequency of 52%. The respective percentages were 46% and 63% for amateur athletes and sedentary participants. All the numbers of the genotypes and alleles are summarized in Table 2.

Discussion

Studies trying to associate athletic performance and genetic parameters showed us that several genes are involved in determining athletic performance. In order to find the optimal genetic endowment, we need to develop genetic models. We need to find if the genetic polymorphisms is advantageous for athletic performance or not. Most studies, therefore, are conducted on these genetic markers, *ACE* ID and *ACTN3* R577X polymorphisms.

ACE has been widely well examined in many athletes and different sport types in the terms of predisposition of gene on athletic performance. Most studies suggest that participants who have "I" allele have more endurance-based activities resulting from the decreased ACE activity whereas participants who have "D" allele have increased power of anaerobic dominated activities resulting from ACE gene expression (13).

In our cohort, we examined 23 national athletes, 27 amateur athletes and 34 sedentary individuals. We divided national and amateur athletes into three groups according to the distance they performed. When compared with other genotypes and amateur short distance runners, in *ACE* genotype, short distance runners had DD genotype more frequently. In this regard, DD genotype seems to be advantageous in short distance performance in athletes. In middle distance athletes, II genotype was more frequent in national athletes, and ID genotype was more frequent in amateur middle distance athletes. ID and II genotype were equal to each other in national long distance athletes, but when we examine amateur athletes, DD genotype was more frequent in long distance runners.

Papadimitriou et al. (2016) compared the sprint times (200m and 400m) and *ACE* genotypes of elite male athletes. In 200m, athletes who had *ACE* DD genotypes had shorter personal sprint times than those with *ACE* II genotype. In 400m, no statistically significant relationship between *ACE* genotype and sprint times was detected. Athletes who had at least one "D" allele (DD and ID genotypes) reported shorter personal sprint times than the athletes with *ACE* II genotype. When they compare *ACE* DD and ID genotypes and 400m athletes in the terms of sprint times, no significant difference was reported. This result is in agreement with our findings, indicating the effect of DD genotype in power- sprint performance. Scott et al. (2010) analysed ACE genotypes of 114 Jamaican and 113 United States sprinters; and reported that ID polymorphism does not play a key role in being an elite athlete. Çam et al (2005) reported the *ACE* ID genotype of 32 female non-elite Turkish athletes and compared their genotype with sprint and middle distance running times. In their cohort, they reported that there were no significant differences amongst *ACE* genotype groups with respect to their sprint or middle distance performances (15). Another study investigated the association between *ACE* ID polymorphism and short and medium-duration aerobic endurance performance improvements in response to the same training regimen in a non-elite 55 female Turkish athletes (16). They concluded that *ACE* II may be related with medium-duration aerobic endurance performance whereas *ACE* DD genotype with more advantageous shorter duration and higher intensity endurance activities. These results indicate the importance of "I" allele with extended performance distance, which is in agreement with our findings.

On the other hand, *ACTN3* is another important genetic marker in the genetics of athletic performance. "R" allele is considered to be the sprinting allele whereas "X" allele is related with endurance athletic capacity. In our cohort, we detected no *ACTN3* RR polymorphism in national athletes. RX genotype was more frequent in

Table 2. *ACE* genotype and allelic distribution of athletes and sedentary participants in the cohort.

	National Athletes (n= 23)				Amateur Athletes (n= 27)				Sedentary Individuals (n= 34)
	Short Distance (n= 12)	Middle Distance (n= 6)	Long Distance (n= 5)	Total	Short Distance (n= 10)	Middle Distance (n= 6)	Long Distance (n= 11)	Total	
DD	6	1	1	8	4	1	6	11	9
ID	3	1	2	6	-	4	3	7	7
II	3	4	2	9	6	1	2	9	18
Alleles									
D	15	3	4	22	8	6	15	29	25
I	9	9	6	24	12	6	7	25	43

all groups. Power- sprint related "R" allele is more frequent in amateur athletes and in sedentary individuals, whereas endurance related "X" allele in national athletes.

Yang et al. (2017) compared 59 elite sprint / power and 44 endurance athletes with 50 healthy controls in the terms of *ACTN3* R577X polymorphism. In their study, authors showed the effect of competition and lower limb strength in elite Chinese sprint/power athletes. They also reported that *ACTN3* RR genotype was much more higher in sprint/power athletes. Eynon et al. (2013) reported three cohorts of European team-sport athletes, including 305 long distance endurance athletes, 378 short and medium distance sprint/power athletes from Poland, Russia and Spain, and team athletes from 205 different branches and 568 non-athletic European men. In their cohort, *ACTN3* R577X polymorphism has been found not to be significantly different when compared to endurance athletes and non-athletic groups in those interested in team sports. However, they reported that 577RR genotype was represented higher in sprint / power athletes. Kikuchi et al. (2016) analysed *ACTN3* R577X polymorphism in 1057 Japanese elite athletes (627 sprint/power sports and 430 endurance sports) and reported an association between *ACTN3* R577X polymorphism and Japanese elite athletes, RR and RX genotypes were associated with both sprint/power and endurance performances. Kim et al. (2014) found that ACTN3 R577X has an important key role in distinguishing sprint performance of *ACTN3* R577X polymorphism in a study conducted by Korean participants (58 sprinter, 63 long-distance endurance athletes and 854 healthy control groups). Authors reported that *ACTN3* R577X gene variant is one of the main important genes for sprint performance, though not every time for power performance. Our results differed in some ways from the studies mentioned. In our cohort, RX genotype in more frequent in national athletes, and we detected no RR genotype. Small subject numbers may be the main reason for this controversial results.

When we consider Turkish subjects, we have limited information regarding *ACTN3* R577X polymorphism in athletes. Sanlisoy et al. (2011) examined the Turkish-Aegean region elite athletes and reported a significant difference between elite athletes and sedentary individuals in the terms of RR and RX genotypes, as well as of R and X alleles. Günel et al. (2014) investigated the effects of *ACE* ID and *ACTN3* R577X polymorphisms on sport performance in 37 elite athletes and 37 healthy controls and concluded that *ACE* ID and *ACTN3* R577X polymorphisms have been shown to be important biomarkers that have an effect on muscle strength. Ulucan et al. (2014) compared the 50m sprinting times and *ACTN3* genotype of trained and untrained middle school children and concluded that R allele is a factor for lower sprint times. They reported that differences were statistically significant and ecologically meaningful in their cohort.

Sport genomics is gaining great importance nowadays, and information we have is getting more by time (24). In the present study, small sample size is the main imitating criteria. Although *ACTN3* and *ACE* are the most widely examined studied genes for sports genetics, we still have limited studies regarding Turkish athletes. But the athletes we examined are from the same team, which can minimize the environmental factors such as training and mental coaching.

References

1. Lortie G, Bouchard, C, Leblanc C, Tremblay A, Simoneau JA, Thériault G et al. Familial similarity in aerobic power. Hum Biol 1982;54:801-12.
2. Corak A, Kapıcı S, Sercan C, Akkoc O, Ulucan K. A pilot study for determination of anxiety related SLC6A4 promoter "S" and "L" alleles in Turkish athletes. Cell Mol Biol (Noisy le Grand) 2017; 63(5): 29-31.
3. Yang N, MacArthur DG, Gulbin JP, Hahn AG, Beggs AH, Easteal S et al. ACTN3 genotype is associated with human elite athletic performance. American Journal of Human Genetics 2003;73:627–31.
4. Ulucan K. Literature Review of Turkish Sportsmen in Terms of ACTN3 R577X Polymorphism. Clin Exp Health Sci 2016;6(1):44-7
5. North KN, Yang N, Wattanasirichaigoon D, Mills M, Easteal S, Beggs AH. A common nonsense mutation results in alpha-actinin-3 deficiency in the general population. Nature Genetics 1999;21:353-4.
6. Ahmetov II, Fedotovskaya ON. Current progress in sports genomics. Adv Clin Chem 2015;70:247-314.
7. Ulucan K, Göle S, Altindas N, Güney A. Preliminary Findings of α -Actinin-3 Gene Distribution in Elite Turkish Wind Surfers. Balkan J Med Genet 2013;16(1):69-72.
8. Papadimitriou D, Lucia A, Pitsiladis YP, Pushkarev VP, Dyatlov DA, Orekhov EF, et al. ACTN3 R577X and ACE I/D gene variants influence performance in elite sprinters: a multi-cohort study. BMC Genomics 2016;17:285.
9. Santiago C, González-Freire M, Serratosa L, Morate FJ, Meyer T, Gómez-Gallego F, et al. ACTN3 genotype in professional soccer players. Br J Sports Med 2008;42(1): 71-3.
10. Guney AI, Ergec D, Kirac D, Ozturhan H, Caner M, Koc G, et al. Effects of ACE polymorphisms and other risk factors on the severity of coronary artery disease. Genet Mol Res 2013;12(4):6895-906.
11. Zhang B, Tanaka H, Shono N, Miura S, Kiyonaga A, Shindo M, et al.
The I allele of the angiotensin-converting enzyme gene is associated with an
increased percentage of slow-twitch type I fibers in human skeletal muscle. Clin Genet 2003; 63(2):139-44.
12. Cieszczyk P, Krupecki K, Maciejewska A, Sawczuk M. The angiotensin converting enzyme gene I/D polymorphism in Polish rowers. Int J Sports Med 2009;30(8):624-7.
13. Sercan C, Eken BF, Erel S, Ülgüt D, Kapıcı S, Ulucan K. Spor Genetigi ve ACE Gen İlişkisi. Journal of Physical Education and Sport Sciences 2016;3(2):26-34.
14. Scott RA, Irving R, Irwin L, Morrison E, Charlton V, Austin K, et al. ACTN3 and ACE Genotypes in Elite Jamaican and US Sprinters. Med Sci Sports Exerc 2010;42(1):107-12.
15. Cam S, Colakoğlu M, Colakoğlu S, Berdeli A. The ACE I/D gene polymorphism and physical performance in a non-elite female cohort. Turkish Journal of Sports Medicine 2005;40(1):1-8.
16. Cam S, Colakoglu M, Colakoglu S, Sekuri C, Berdeli A. ACE I/D gene polymorphism and aerobic endurance development in response to training in a non-elite female cohort. J Sports Med Phys Fitness 2007; 47(2):234-8.
17. Yang R, Shen X, Wang Y, Voısın S, Caı G., Fu Y, et al. ACTN3 R577X gene variant is associated with muscle-related phenotypes in elite chinese sprint/power athletes. Journal of Strength and Conditioning Research 2016; 31(4):1107–15.

18. Eynon N, Banting LK, Ruiz JR, Cieszczyk P, Dyatlov DA, Maciejewska-Karlowska A, et al. ACTN3 R577X polymorphism and team-sport performance: a study involving three European cohorts. J Sci Med Sport 2014; 17(1):102-6.
19. Kikuchi N, Miyamoto-Mikami E, Murakami H, Nakamura T, Min SK, Mizuno M. ACTN3 R577X genotype and athletic performance in a large cohort of Japanese athletes. Eur J Sport Sci 2016;16(6):694-701.
20. Kim H, Song KH, Kim CH. The ACTN3 R577X variant in sprint and strength performance J Exerc Nutrition Biochem 2014;18(4):347-53.
21. Sanlısoy F, Altıntas N, Buyukyazı G, Candan N. Investigation of ACTN3 R577X genotype distribution of elite athletes in the Aegean region. Cumhuriyet Med J 2011;33:153-9.
22. Günel T, Gümüşoğlu E, Hosseını MK, Yılmazyıldırım E, Dölekçap I, Aydınlı K. Effect of angiotensin I-converting enzyme and α-actinin-3 gene polymorphisms on sport performance. Molecular Medıcıne Reports 2014;9:1422-6.
23. Ulucan K, Bayyurt GM, Konuk M, Güney AI. Effect of alpha-actinin-3 gene on trained and untrained Turkish middle-school children's sprinting performance: a pilot study. Biological Rhythm Research 2014;45(4):509–14.
24. Eroğlu O, Zileli R. The Effect Of Genetic Factors On Sportive Performance. IJSETS 2015;1(1):63-76.