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Original article

Determination of the distribution angiotensin-converting enzyme (ACE I/D) and alpha-actinin-3 (ACTN-3 R577X) among elite sprinters and middle-long distance runners in Turkey

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Abstract. *Study Objectives:* The purpose of this study was to define ACTN3 and ACE genes affecting the athletic performance of sprinters and mid-long distance athletes. *Methods:* A total of 184, elite sprinters and mid-long distance athletes and sedantary participated in the study. Subjects were categorized into three groups as sprinters, mid-long distance athletes, and sedantary. After DNA isolations, the Single PCR technique was used for ACE genotyping and Real Time PCR was used for ACTN3 genotyping. *Results:* The R allele of the ACTN3 gene was found to be high for sprinters. The allele of ACE D gene was high for distance athletes. *Conclusion:* ACTN3 and ACE genes allele distributions of sprinters and distance athletes were determined in relation with speed for the R allele of ACTN3 and with endurance for the D allele of the ACE gene.

Key Words: Sprinter, Mid-Long Distance Runners, ACTN3, ACE

Introduction

Sporting performance is a combination of genetic and phenotype characteristics. In addition to characteristics comprising physical performance like speed, power, endurance, flexibility, and coordination, psychological traits like concentration, self-confidence, ability to deal with stress, and motivation are determined by genetic infrastructure (1). Sports genetics researches the effect of genetic variations affecting the athletic performance of athletes on characteristics determining athletic performance. Determination of these variations is important to direct sporting candidates to the sports branch they are skilled at a young age. This scientific ability selection method will make it easier to choose sports for both sporting candidates and sports clubs.

There are over 200 gene studies about sports genetics (2). Of these genes, 2 genes have been intensely studied for their relationship with sporting performance, especially. These genes are angiotensin-converting enzyme (ACE I/D) and α-actinin-3 (ACTN3 R577X). The ACE gene spans 21 kilobases, is located on the 17th chromosome q23, and consists of 26 exons and 25 introns. The most common mutation of ACE is I (insertion) / D (deletion) polymorphism. The ACE gene has DD, ID, and II alleles. A 287-bp fragment causes important changes in the

ACE level, with presence forming insertion (I allele) polymorphism and absence forming deletion (D allele) polymorphism. In serum and tissue, individuals with the DD genotype of the ACE gene are most common, while individuals with the II allele are less frequently encountered (3,4). The ACE gene has duties in the cardiovascular system. People with high ACE levels have excess Ang II production and excess Ang II causes vasoconstriction (narrowing of veins). This causes a reduction in perfusion to muscles (5). This situation is thought to lead to low aerobic endurance characteristics in parallel with the reduction in oxygen reaching muscles in individuals with the DD allele. When studies related to the ACE gene were examined, many studies have reported that the I allele of the ACE gene is dominant in endurance-type sports (6-8) and that the D allele is dominant in speed, power, and strength sports (9,10). However, there are also studies stating that the allele and genotype of the ACE gene are not associated with athletic performance (11,12).

The other gene related to athletic performance is the ACTN3 (ACTN3 R577X) gene. This gene has 3 genotypes of RR, RX, and XX. In ACTN3 R577X polymorphism, the C→T transition occurring in the 1747 position on the 16th exon transforms the codon forming arginine (R) amino acid at 577 positions into a stop codon (X). However, α-actinins are members of the actin-binding protein family (dystrophin, spectrin, filamin, and fimbrin) forming and regulating the cellular skeleton and are basic structural components of the Z line in skeletal muscles (13). This interaction ensures the relationship between structural and signal proteins with the Z line. Especially, skeletal α-actinin and sarcomeric α-actinins (-2 and -3) preserve the static function of the myofibrillary array and regulate myofibrillary contraction (14). If the X allele is homozygotic (XX genotype) α-actinin 3 is not synthesized. R577X is associated with sporting status and human muscle performance. The deficiency of α-actinin 3 affects the function of fast-twitch fibers. Deficiency of α-actinin-3 may be resolved by the ACTN2 isoform without causing pathologic phenotypes like muscular dystrophy or myodystrophy. If individuals have at least one copy of the R allele, the ACTN3 gene produces sufficient α-actinin-3. If α-actinin-3 had a significant effect on type II muscle fibers, differences in skeletal muscle functions may be compared between individuals with different ACTN3 genotypes (R577X). As a result, individuals with certain ACTN3 gene (RR or RX genotype) are expected to be more successful at sports requiring strength and power compared to individuals with XX genotype. When the literature is examined, there are studies stating the R allele is dominant in speed and power sports (8,15-18). In this context, the aim of our study was to investigate the distribution of ACTN3 and ACE genes affecting athletic performance in Turkish sprinters and mid-long distance runners.

Materials and Method

Participants

Forty-four elite Turkish athletes participated in the study. Of these athletes, 26 were sprinters (100 m, 200 m and 400 m) and 18 were mid-long distance runners (5000 m and 10000 m). One hundred and forty sedentary individuals participated in this study as a control group. These athletes participate in national and international competitions and were licensed in the highest league of their sports branch. The descriptive characteristics of the subjects were given in Table 1. After all, subjects were informed about the study, they agreed to voluntarily participate. The necessary ethics committee permission was obtained the Marmara University Faculty of Medicine ethics committee (protocol no: 09.2016.175).

Determination of ACTN-3 Genotype Distribution

This process was completed with 0.5 ml, Eppendorf tubes and tubes were inserted in a real-time device with the chosen program applied. The RT program for the ACTN3 r577x allele region was 95 °C for 600 s Hold, then 95 °C for 15 s (2- Step Amplification), and then 60 °C 60 s (2- Step Amplification-reading).

Determination of ACE Genotype Distribution

For this process 0.5 ml Eppendorf tubes were used and tubes were inserted into a heat cycle device and the selected program was applied. After pre-denaturation for 3 minutes at 95 °C, then 45 s denaturation at 95 °C, then 1 min matching at 58 °C, the 45 s synthesis at 72 °C, and then 7 min final stage at 72 °C were applied. After the polymerase chain reaction, the amplicons for the exon 26 region were investigated with 2% agarose gel electrophoresis.

Agarose Gel Electrophoresis

For the identification of products reproduced with PCR, 2% agarose gel was prepared. For this, 0.7 g agarose was dissolved in 35 ml 1X TAE and boiled in a microwave oven. The solution had 2 μg/ml ethidium bromide (EtBr) added to be able to image DNA under UV light. An appropriate grid was inserted into the gel mold to create the necessary number of wells and the gel was poured into the mold and left at room temperature until well-polymerized. PCR products of 10 μl were mixed with 2 μl loading buffer and placed in the wells. Electrophoresis of 100 V/40 mA was applied. After nearly 30 minutes, the PCR bands illuminated under UV light due to ethidium bromide were observed and compared with standard markers.

Statistical Analysis

The data was analyzed with the SPSS 20 package program. Descriptive statistics of age, height, weight, body mass index (BMI), and athlete age were presented as a minimum, maximum, and mean. A chi-squared test was used to confirm that the observed genotype frequencies.

Results

When Table 1 was examined, the age, height, body weight, body mass index, and sportive experience of sprinters are as follows; 19.68 ± 2.33 years (age), 1.72 ± 6.89 cm (height), 62.50 ± 9.06 kg (weight), 21.52 ± 2.02 (BMI), 5.70 ± 2.44 (sportive experience). The age, height, body weight, body mass index, and sportive experience of mid-long distance runners are as follows; 18.72 ± 2.62 years (age), 1.67 ± 7.06 cm (height), 60.13 ± 9.68 kg (weight), 21.83 ± 2.22 (BMI), 5.50 ± 1.92 (sportive experience).

Within the scope of the study, 44 sprinter and mid-long distance runners participated. Of the subjects, 21 were female and 33 were male. The genotype and allele frequency distribution for the ACE gene region is given in Table 2. Of sprinters, 8 had DD, 10 had DI and 8 had II genotype. When the allele distribution of sprinters is investigated, 50% had D allele

Table 1. Descriptive Characteristics of Subjects

Variables	Sprinters Mean±Std. Deviation	Mid-Long Distance Runner Mean±Std. Deviation	Control Mean±Std. Deviation
Age (year)	19.68 ± 2.33	18.72 ± 2.62	20.02 ± 3.33
Height (m)	1.72 ± 6.89	1,67 ± 7,06	1,69 ± 5.08
Weight (kg)	62.50 ± 9.06	60.13 ± 9.68	64.22 ± 8.12
Body Mass İndeks (BMI)	21.52 ± 2,02	21.83 ±2.22	22.48± 2.1
Sportive Experience (year)	5.70 ±2.44	5.5 ± 1.92	-

and 50% had I allele. When the genotypes of mid-long distance runners are examined, 8 people had DD, 8 people had DI and 2 people had II genotypes. When the allele distribution of mid-long distance runners is examined, 67% had D allele and 33% had I allele. Of control; 40 had DD, 65 DI, and II 35 genotype. Allele distribution of control; 50% had D allele and %50 had I allele. D allele frequency was higher than I allele frequency in mid-long distance runner (p=0.46). No significant allele frequency height was found in other groups (p>0.05; Table 2).

The genotype and allele frequency for ACTN3 is summarized in Table 3. Of sprinters, 12 had RR, 8 had RX and 6 had XX genotype. When the allele distribution of sprinters is investigated, 62% had R and 38% had I alleles. For mid-long distance runners, there were 5 RR, 8 RX, and 5 XX genotypes. When the allele distribution of mid-long distance runners is investigated, 50% R allele and 50% X allele was determined. Of control; 27 had RR, 36 RX and XX 42 genotype. Allele distribution of control; 40% had R allele and %60 had X allele. R allele frequency was higher than the X allele frequency in control (p=0.007). No significant allele frequency height was found in other groups (p>0.05; Table 3).

Discussion

At a certain level, athletes competing nationally and internationally in their branch are called elite athletes (19). To display superior athletic performance at elite levels, the maximum performance of aerobic and anaerobic energy systems is required according to the sportive branch. This performance is shaped by genetic and environmental effects. Related to genetics, in recent years two genes, especially, have been associated with athletic performance. These genes are ACTN3 and ACE genes. In our study, the distribution of ACTN 3 and ACE polymorphism distribution, proposed to affect athletic performance in previous studies, were investigated in elite sprinters and mid-long

Table 2. Genotype and Allele Distribution for Angiotensin-Converting Enzyme Chi-Square test

	n	ACE Genotype			Chi-Square		Allele Frequency		Chi-Square	
		DD	DI	II	X^2	p	D	I	X^2	P
Sprinters	26	8 (%31)	10 (%38)	8 (%31)	0.30	0.85	26 (%50)	26 (%50)	0	1
Mid-Long Distance Runners	18	8 (%44)	8 (%44)	2 (%12)	1	0.60	24 (%67)	12 (%33)	4	**0.46***
Control	140	40 |(%28)	65 (%46)	35 (%26)	3.25	0.19	135 (%50)	135 (%50)	0	1

Table 3. Genotype and Allele Distribution for Alpha Actinin-3 Chi-Square test

	n	ACTN 3 Genotype			Chi-Square		Allele Frequency		Chi-Square	
		RR	RX	XX	X^2	p	R	X	X^2	p
Sprinters	26	12 (%46)	8 (%31)	6 (%23)	2.15	0.34	32 (%62)	20 (%38)	2.76	**0.05***
Mid-Long Distance Runners	18	5 (%28)	8 (%44)	5 (%28)	4	0.13	18 (%50)	18 (%50)	0,00	1
Control	106	27 (%24.5)	36 (%34.5)	42 (%40)	3.25	0.19	80 (%40)	118 (%60)	7.29	**0.007***

distance runners from Istanbul. When the ACE (D/I) results of sprinters and mid-long distance runners participating in the study were examined, sprinters had an equal distribution of 50% D allele (deletion) and 50% I allele (insertion). When the ACE results of mid-long distance runners were investigated, there was a 67% D allele and 33% I allele found. In our study, the D allele was dominant compared to the I allele in mid-long distance runners. The I allele of the ACE gene was stated to be dominant in long-duration endurance sports (20), while the D allele was stated to be dominant in short-duration sprinting and power sports in previous studies (21,22). Ma (2013) assessed a total of 366 articles in a meta-analysis study about ACE and ACTN3. According to the results, the ACE II genotype is associated with endurance while the ACTN3 R allele is associated with sprint performance (8). When studies investigating the sprint traits of the ACE gene were examined, Myosotis found the distribution of the D allele (69%) was proportionally greater than the I allele (31%) in a study of 12 sprinters (10). Cam (2005) studied 88 Caucasian sprinters and found the distribution of the D allele was more intense (9). Scott et al. (2010) found no correlation between the ACE gene and sprinting in a study of 230 Jamaican and American sprinters (23). Our study results cannot be used to examine the sprint traits of ACE gene polymorphism. Collins (2004) associated endurance with the I allele in a study of North African triathlon athletes (7). Rankinen et al. (2000) in a study of 192 endurance sportspeople found the I and D alleles of the ACE gene were not associated with endurance sport (11). Magi et al. (2015) examined Estonian male skiers and found no correlation of endurance with the ACE I/S gene (12). Akalın (2013) found the D allele was proportionally found more often compared to the I allele among Turkish long distance runners (24). Amir (2007) found that the D allele was higher among endurance athletes in a study of Israeli marathon runners (25). In our study, the D allele was found more often than the I allele of the ACE gene in mid-long distance runners.

When the ACTN3 results of our study were examined, the R allele (62%) was found more often among sprinters compared to the X allele (38%). Ulucan (2016) studied 234 Turkish athletes from different branches and found the R allele frequency of the ACTN3 gene was more common (18). Yang et al. (2003) found the R allele was more common (72%) in a study of elite sprinters (26). Druzhevskaya et al. (2008) studied 486 male and female Russian power athletes (volleyball, sprint, bodybuilding, football, wrestling) and found the frequency of the R allele was higher (17). Eynon et al. (2010) found the R allele frequency was higher among 81 Israeli sprinters (16). However, there are studies stating that the ACTN3 gene is not associated with sprint performance. Scott et al. (2010) in a study of 230 Jamaican and American sprinters found ACTN3 and sprinting were not correlated (23). Kikuchi et al (2015) found no significant correlation between athletic performance and the ACTN3 gene in a study of 253 Japanese college athletes (27). Kim et al. (2014) found a correlation between the ACTN3 gene XX genotype and sprint performance for Korean sprinters (28).

When the correlation of ACTN3 with endurance was investigated in our study, there was no correlation between the R and X allele frequency distribution and endurance athletes. When this result was compared with the literature, a study by Niemi and Majamaa (2005), 52 Finnish endurance athletes found the XX genotype was more common among endurance athletes (29). Ahmetov et al. (2010) found a significant relationship between XX genotype and endurance athletes in a study of Russian athletes (30). Saunders et al. (2007) did not find a correlation between ACTN3 and endurance performance in a study of endurance athletes (20). Eynon et al. (2010) investigated the correlation between R and X allele distribution of ACTN3 with endurance in Israeli athletes and found no correlation between R and X alleles and endurance (16).

Conclusion

The R allele of the ACTN3 gene in sprinters and the D allele of the ACE gene in Mid-long distance runners were found to be high in our study. These results support the R allele exists more in sprinters in the literature. In contrast to the I allele exists more in the literature in Mid-long distance runners in the ACE

gene, the D allele was found to be higher. The R allele of the ACTN 3 gene in sprinters and the D allele of the ACE gene in Mid-long distance runners should be considered in the evaluation of athletic performance.

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Conflicts of Interest

The authors declare that there is no conflict of interest in this manuscript.

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