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The Relationship among the performance and the *ACE* I/D and *ACTN3* R577X polymorphisms and some anthropometric factors in arm wrestlers

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ABSTRACT

Sport genetics has become increasingly important in recent years. The concept of performance includes genetic and anthropometric factors. These factors have not been adequately examined when the current literature is examined. One of the aims of this study is to identify a possible interaction between the ACE ID and ACTN3 R577X polymorphisms and the sedentary, national arm wrestlers and amateur arm wrestlers, while another aim is to reveal some physical differences between the same persons. The research included 24 arm wrestlers who were members of the Turkish national team, 23 amateur arm wrestlers with club licenses, and 34 sedentary, all of the wrestlers participated voluntarily in the research. The genotype distribution of the ACE gene and ACTN gene and the statistical significance of the R, X, I, and D allele frequencies were compared by the Chi-Square test. The Anova one-way variance analysis was used to assess the difference among the palm circumference, wrist circumference, and forearm width among the groups, the significance was tested at $p<.05$ level. After the data was evaluated, significant differences were not found statistically in ACE nor ACTN3 polymorphisms in terms of the three groups ($p>0.05$). In addition, statistically significant differences were found in the palm, wrist, and forearm circumferences of the arm wrestlers who were the members of the Turkish national team compared especially to the sedentary ($p<0.05$). From the conducted research it was concluded that the success of the athlete in arm wrestling could not be directly explained by the *ACE* and *ACTN3* genotypic characteristics. Another result revealed by this study was that the success of the athlete in arm wrestling was more related to palm, wrist, and forearm circumferences.

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Introduction

Arm wrestling is a simple type of sport where the winner and loser are usually identified in a very short period. The elbows of the competitors who hold each other's palms are mutually placed on a solid surface, the fight ends when one of the players pushes the rival's arm on the surface after the competition was started (1). In this sport branch, both of the arm wrestlers must expend maximum power with a single arm to win the competition.

In sports branches such as arm wrestling and weight lifting, the muscles contract isometrically and the brachialis muscle gets the elbow to flex (2). This muscle is located between the humerus and ulna. The biceps brachia muscle, which is on the brachialis muscle gets the arm to flex and supine. The upper two heads of this muscle are in the scapula bones while the lower two heads are in the radius and ulna bones. In arm wrestling, both muscles have functions (3).

Success in arm wrestling can be determined by internal and external factors, such as physical, physiological, psychological and environmental factors (4). Arm wrestling has a structure that requires maximum muscle strength and high levels of anaerobic performance (5,6).

Factors such as age, gender, anatomical structure, intelligence, state of the locomotor system, psychological balance, autonomic nervous system, functions of the secretory glands, metabolism, energy utilization mechanisms, state of the organ systems, neuromuscular transmission rate, cardiovascular system, and especially the genetic endowment of players are the other important factors that are crucial for athletic performance (7). There is a strong belief that the success of elite athletes is due to the effects of the genotype combination. Recent studies have reported that there are approximately 240 gene variants considered to be potentially related to athletic performance at the elite level but only a very few of them are really associated with athletic performance (8). *ACE* InDel and *ACTN3* rs1815739 polymorphisms are among the most widely examined polymorphisms of elite athletes. The variants of these genes have been reported to be associated with the physical performance characteristics of elite-level athletes. Some of the studies related to these polymorphisms were conducted on Jamaican and American sprinters (9), Japanese elite wrestlers (10), well-trained endurable athletes (11), Brazilian footballers (12), Lithuanian

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athletes (13) and Polish swimmers (14), Turkish athletes and soccer players (15,16) and controversial results were reported.

In the present study, we aimed to determine a possible interaction between the *ACE* InDel and *ACTN3* rs1815739 polymorphisms of sedentary, amateur arm wrestlers and national arm wrestlers, and reveal the anthropometric differences between the wrestlers at different levels.

Materials and Methods

Participants

24 National arm wrestlers, 23 amateur arm wrestlers and 34 sedentary individuals (as a control group) were enrolled in the study. Osmangazi University, Ethical Committee of Clinical Investigations of Medical Faculty approved the study protocol (2016/22), and the study is conducted in accordance with the principles of the Declaration of Helsinki II. Written informed consent that explains the study steps and aims was signed by all participants. This work was supported by Bilecik Şeyh Edebali University Scientific Research Organization (2015-02.BSEÜ.13-01).

Anthropometric Measurements

All measurements were done using an anthropometric tape measure (Gullick Meter) with 1mm ± sensitivity and recorded in cm. The circumference of the wrist was measured at the joint of the radius and the ulna which corresponds to the perimeter of the thinnest part of the forearm. The circumference of the hand was measured as the perimeter of the middle part of the hand, located over the two major transverse palmar creases "heart line" and "head line" (17).

Genotyping

A commercially available DNA isolation Kit (MACHEREY-NAGEL NucleoSpin® Blood) was used for the isolation of DNA samples from peripheral blood samples. Conventionally spectrophotometer values of A260/A280 were used to determine the purity of the isolates. DNA samples were kept at -20 C˚ until used for genotyping. Polymerase chain reaction-restriction fragment length polymorphism (PCR- RFLP) methodology was used for *ACTN3* rs1815739 genotyping. Forward, 5'-CTG TTG CCT GTG GTA AGT GGG-3' and reverse, 5'-TGG TCA CAG TAT GCA GGA GGG-3', primers used for amplification. For PCR, 35 cycles were performed, initial denaturation at 95°C for 5 min., followed by denaturation at 95°C for 30 seconds, annealing and extension at 72°C for 1 min., and a final extension for 7 min. at 72°C was used. Amplified 290 bp amplicons were digested by DdeI (New England Biolabs, Beverly, MA, USA), as recommended by the manufacturer. Digested fragments were separated on 3% agarose gel electrophoresis and visualized under UV light by ethidium bromide staining. The wild-type allele, 577R, showed fragments of 205 and 85 bp, whereas the variant allele, 577X, showed fragments of 108, 97 and 85 bp.

ACE genotyping was performed by conventional PCR reaction. Primers 5'-CTGGAGAC-CACTCCCATCCTTTCT-3' and 5'-GATGTGGCCATCA-CATTCGTCAGT-3' were used for the amplification. PCR mixture contained 50- 100 ng of isolated DNA, 50 mM KCl, 1 mM dNTP, 1.5 mM MgCl2, 10 mM Tris-HCl, pH 8.0, and 1.5 U Taq DNA polymerase. PCR conditions were as follows: initial denaturation at 94°C for 5 min; annealing at 58°C for 1 min, and extension at 72°C for 2 min. After the 1st cycle, 30 cycles were performed: denaturation at 94°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 2 min. following the completion of cycles, a final step is followed: 94°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 7 min. PCR amplicons were separated by electrophoresis on a 2% agarose gel and visualized under ultraviolet light after ethidium bromide staining. Genotyping was completed by having a 490-bp band (II genotype), a 190-bp band (DD genotype), or both 490- and 190-bp bands (I/D genotype).

Statistical analysis

Chi-square analysis (SPSS 22 for windows, SPSS Inc., Chicago, IL., USA) was used to determine the significant difference between the genotypes of the groups. p values that are less than 0.05 were considered as significant. To determine the statistically significant differences between hand, wrist and forearm circumferences, we used the one-way ANOVA test (SPSS 22 for windows, SPSS Inc., Chicago, IL., USA). The Bonferroni test was used to clarify the results.

Results

All the players and sedentary individuals were successfully genotyped. For *ACE* InDel polymorphism, 14 (58,3%) of the 24 National players had II genotype, 2 (8.3%) had ID and 8 (33.3%) had DD genotypes. For amateur players, 11 (47.8%), 4 (17.4%) and 8 (34.8%) players had DD, ID and II genotypes, respectively. For the sedentary individuals, the respective numbers and percentages were 18 (52.9%), 9 (26.5%), and 7 (20.6) for II, DD, and ID genotypes. No statistically significant difference was

Table 1. *ACE* InDel genotypes of players and sedentary participants in the study cohort. The p-value represents the statistical difference between study groups.

	***ACE* Genotype**				
Groups	**II**	**ID**	**DD**	**Total**	**p**
Sedentary	18 %52.9	7 %20.6	9 %26.5	34	0.325
Amateur Arm Wrestlers	8 %34.8	4 %17.4	11 %47.8	23	
National Arm Wrestlers	14 %58.3	2 %8.3	8 %33.3	24	

Significant at p<0.05 level.

detected between the groups in the terms of *ACE* InDel polymorphism (Table 1).

For ACTN3 rs1815739 polymorphism, 7 (27.2%) of the players had RR, 13 (55.6%) had RX and 4 (17.3%) had XX genotypes, whereas 6 (26.1) of the amateur players had RR, 15 (56.2%) had RX and 2 (8.7%) had XX genotypes. When we compare the genotypes of ACTN3 rs1815739 polymorphism in our cohort, we detected no statistically significant difference (Table 2).

The average hand circumference of our cohort was 34.04, 43.53 and 48.93 cm for sedentary, amateur and national players, respectively. For statistical analysis, we analysed them in 3 groups; national players with amateurs, nationals with sedentary and amateurs with sedentary. We detected no statistically significant difference between them, only detected a significant difference between national players and sedentary individuals (Table 3).

For wrist circumferences, we had an average of 16.16, 17.32 and 18.32 cm for sedentary, amateurs and national arm wrestlers, respectively. When we compare them with each other, we had a significant difference between national arm wrestlers and sedentary (Table 4).

For forearm circumferences, respective average lengths were 24.81, 27.52 and 30.22 cm for sedentary, amateurs and national arm wrestlers. when we compare sedentary with amateurs, amateurs with nationals, and sedentary with nationals, we all had a statistically significant difference (Table 5).

Discussion

In this study, the distribution of the two widely analysed gene variants (*ACE* InDel and *ACTN3* rs1815739 polymorphisms) and the difference in some physical pa-

Table 2. *ACTN3* rs1815739 genotypes of our study cohort. The p-value represents the statistical difference.

	ACTN3 Genotype				
Groups	**RR**	**RX**	**XX**	**Total**	**p**
Sedentaries	9 %26.35	17 %50	8 %23.5	34	0.665
Amateur Arm Wrestlers	6 %26.1	15 %56.2	2 %8.7	23	
National Arm Wrestlers	7 %27.2	13 %55.6	4 %17.3	24	

Significant at p<0.05 level.

Table 3. The average hand circumference (in cm), standard deviation (sd), f and p values of sedentary, amateur and national arm wrestlers.

	$\bar{x}$	**sd**	**f**	**p**
Sedentary & Amateur Arm Wrestlers	34.04 43.53	13.53 12.60	7.353	0.100
Amateur Arm Wrestlers & National Arm Wrestlers	43.53 48.93	12.60 11.60		0.448
Sedentary & National Arm Wrestlers	34.04 48.93	13.53 11.60		0.001*

*Significant at p<0.05 level, $\bar{x}$: arithmetic mean, sd: standard deviation.

Table 4. The average wrist circumferences (in cm), standard deviation (sd), f and p values of sedentary, amateur and national arm wrestlers.

	$\bar{x}$	**sd**	**f**	**p**
Sedentary & Amateur Arm Wrestlers	16.16 17.32	1.51 2.33	11.623	0.070
Amateur Arm Wrestlers & National Arm Wrestlers	17.32 18.54	2.33 1.72		0.078
Sedentary & National Arm Wrestlers	16.16 18.32	1.51 1.72		0.000*

*Significant at p<0.05 level, $\bar{x}$: arithmetic mean, sd: standard deviation.

Table 5. The average circumference of the forearm (in cm), standard deviation, f and p values of sedentary, amateur and national arm wrestlers.

	$\bar{x}$	**Sd**	**f**	**p**
Sedentary & Amateur Arm Wrestler	24.81 27.52	2.46 3.22	22.696	0.004*
Amateur Arm Wrestler & National Arm Wrestler	27.52 30.22	3.22 3.44		0.008*
Sedentary & National Arm Wrestler	24.81 30.22	2.46 3.44		0.000*

*Significant at p<0.05 level, $\bar{x}$: arithmetic mean, sd: standard deviation.

rameters were examined in terms of the sedentary, amateur arm wrestlers and national arm wrestlers. Our results showed that there was no statistically significant in either *ACE* InDel or in *ACTN3* rs1815739 polymorphisms in all three groups ($p>0.05$) (Table 1-2). In all groups, *ACE* II and *ACTN3* RX genotypes were superior to the other genotypes. To date, no study intended to examine the interaction of the *ACE* InDel and *ACTN3* rs1815739 polymorphisms in arm wrestlers and this study is the first related to this issue. Before, no statistically significant difference was reported in research that compared the *ACE* InDel and *ACTN3* rs1815739 polymorphisms with the control groups in Jamaican and American elite sprinters and reported that the *ACE* InDel and *ACTN3* rs1815739 polymorphisms were not determinative in terms of the performance of the elite sprinters (9). In another study, it was reported that the *ACE* InDel polymorphism of the Polish endurable athletes did not play an effective role in their success (11). In research conducted on some Brazilian football players, the *ACE* InDel gene was not found to determine in the performance of the football players (12). However, in a study conducted on Turkish football players, Ulucan et al. (16) emphasized that *ACE* InDel and *ACTN3* rs1815739 polymorphisms could be important genetic markers of becoming a successful football player.

In a study, the athletic performances of the Japanese elite wrestlers were reported to be associated with the combination of the ACE InDel and ACTN3 rs1815739 polymorphisms (10). In addition to this, some of the conducted research showed that ACE InDel polymorphisms were predominate especially in the middle and long-distance swimmers compared to the control groups, and also reported that *ACE* I allele did not reflect the same profile in short-distance swimmers (14,18,19,20). Lisa and colleagues emphasized in their research on the genetic effects on athletic performance that the current findings might be advantageous in achieving sportive performance at the elite level in case the positive genetic profile was combined with the right training (21). In another review, it was argued that genetic factors could affect sports performance and that genetic differences could change sportive performance by changing the phenotypic characteristic of the person (22).

When we consider the anthropometric values, a statistically significant difference was detected in the palm, wrist and forearm circumferences of the arm wrestler at the national level compared especially to the sedentary ($p<0.05$) (Tables 3-5).

In sports branches where the hand is used more heavily, the physical and physiological parameters related to the hand constitute an important element of sportive success. In many sport branches, the physical characteristics of the athletes create the basis of the performance and success criteria. In this conducted study, a significant difference was detected in the measurement of the palm and wrist circumference between the sedentary and the national athletes. Moreover, statistically significant differences were also found between the sedentary and amateur arm wrestlers, the amateur and national arm wrestlers and the sedentary and national arm wrestlers in terms of the forearm's width. In the literature, the number of studies investigating the relationship between arm wrestlers in terms of anthropometric features is rather limited. Unlike the obtained results of this study, Akpınar and colleagues was unable to identify a significant correlation between the performance and the circumference and length of the forearm in the research he conducted on 53 athletes at the Turkey arm wrestling championships in 2012 (23). However, Zileli and colleagues conducted a study of 44 national arm wrestlers and reported that palm width and wrist width values of athletes who became successful in their left and right hand are higher than the palm width values of the athletes who could not become successful (24). Similar results were reported by Akpınar and colleagues their study was on 73 national arm wrestlers at Turkey Championships and showed that players having wider forearm circumferences had more advantages in winning matches. Nevertheless, the same authors conducted a study of 31 national arm wrestlers and reported that the ones having wider forearm environments were more advantageous in the game performance (25). As a result, when they examine the differences between the first three graders and the non-graders, they stated that anthropometric parameters both affect success significantly and they could be used in the selection of the skills as well.

Our results indicate that success in arm wrestling cannot be directly explained by the *ACE* InDel and *ACTN3* rs1815739 polymorphisms. However, it is thought to be important to investigate different gene variants, which are considered to be effective in athletic success. Another result emerging from this study is that palm and wrist circumferences are related to the success of arm wrestlers. An important finding of the study is the fact that the forearm circumference is an important assessment tool. Therefore, the width of the forearm is determinant for sedentary, amateur and professional arm wrestlers. It is thought that the anthropometric characteristics should be evaluated especially for the skill selection and the athletes who are selected in this way may be more successful.

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Conflicts of Interest
The authors have no conflicts of interest to declare.

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