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Association of ACE Gene Polymorphism with Cardiovascular Determinants of Physical Performance in Healthy Iranian Men

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Abstract

Background: *Physical performance phenotypes are formed by the interaction of genetic and environmental factors, and gene polymorphisms can influence physical and athletic abilities. An ACE gene insertion/deletion (I/D) polymorphism has been reported to influence physical performance, but its mechanism remains controversial.*

Methods: *The frequency of this polymorphism in 146 healthy Iranian males was determined. Then, the associations between different ACE genotypes with physical performance factors were investigated for 43 of the 146 participants.*

Results: *The frequencies of DD, ID and II genotypes were 38.5%, 41.5%, and 20%, respectively. Although there were no significant associations between the ACE polymorphisms and physical performance factors, the pulse pressure amplification, post-exercise heart rate, and resting heart rate were significantly different between variants with and without the I allele (P=0.02, 0.04, and 0.05, respectively). Furthermore, the ACE polymorphism was a significant predictor of exercise endurance and ventricular function in multivariate analyses (P<0.05).*

Conclusions: *The ACE polymorphism correlated with cardiovascular determinants of physical performance, rather than musculoskeletal factors. Therefore, the ACE I/D polymorphism could not be utilized as a singular genetic biomarker for the assessment of physical performance in the Iranian population. However, a combination of genetic and cardiovascular biomarkers may determine physical performance capacities.*

Keywords: Physical performance, ACE gene, Polymorphism, Cardiovascular hemodynamics.
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Introduction

Over 20,000 genes contain a myriad of variations that define human phenotypes. Physical and athletic performance is the result of a vast array of phenotypes such as muscle strength, skeletal structure, tendon elasticity, and heart and lung size. Each phenotype is the outcome of complex interactions between different body systems.[1] Previous studies have shown there are many genetic factors that influence physical performance through different human populations. These genes are involved in endurance abilities, muscle performance, tendon apparatus, ligamentous apparatus, and psychological aptitudes.[2] Many polymorphic genes have been reported as genetic markers associated with athletic and physical performance, including angiotensin-converting enzyme (ACE), bradykinin receptor (BDKRB2), α-actinin-3 (ACTN3), creatine kinase (CKM), alpha-2A adrenoceptor (ADRA2A), sodium potassium adenosine triphosphate (Na+-K+-ATPase), peroxisome proliferator-activated receptor alpha (PPARα), peroxisome proliferator-activated receptor-γ coactivator 1α (PPARGC1A), endotelial PAS domain protein 1 (EPAS1), leukocyte antigens, and mitochondrial DNA (mtDNA) have been mentioned most frequently in the literature.[3]

The ACE gene has been reported to be an influential factor on human physical performance.[4] This gene encodes the ACE enzyme, which plays an important role in the renin-angiotensin system (RAS) pathway, which controls the homeostasis of the human circulatory system. ACE converts angiotensin I to angiotensin II, which acts as a potent vasoconstrictor. ACE hydrolyzes bradykinin, a vasodilator, so it reduces peripheral resistance, which consequently leads to lower blood pressure.[5] There is a common genetic variation in intron 16 of the ACE gene that consists in the absence (allele D) or presence (allele I) of a 287 bp Alu sequence. This insertion/deletion (I/D) polymorphism (rs1799752) accounts for about half of the variations in serum ACE levels. Allele D is associated with increased levels of ACE in the circulatory system and other tissues.[6,7] Previous studies have shown that, allele I of ACE is associated with endurance-oriented performance, while allele D is related to strength-oriented performance.[8]

The ACE I/D polymorphism has been reported to have an association with physical performance in other populations in the world. Because physical performance could be influenced by individuals' genetic background in different populations, in this study we aimed to determine whether there were any associations between different ACE genotypes, physical performance, and cardiorespiratory fitness in the Iranian population.

Materials and Methods

The participants for this study were randomly recruited from a healthy Iranian male population, ages 20–24 years old, in a military headquarters. In total, there were 146 individuals included in the genotyping phase of the study. Also, 45 of these individuals voluntarily participated in a series of physical performance, cardiorespiratory and cardiovascular evaluation tests. Any history of symptomatic cardiovascular diseases or diabetes mellitus was grounds for exclusion, but no cases of these conditions were detected. Two individuals could not complete their tests due to an episode of ankle sprain that limited their preparedness for the tests. Therefore, the physical

performance data for 43 participants were analyzed. This study was approved by the Research Ethics Committee of Aja University of Medical Sciences. The participants signed an informed consent form prior to taking part in the study.

About 5 ml of peripheral blood was for each person and transferred to a laboratory in EDTA-containing tubes for DNA extraction. The whole genomic DNA was extracted using the High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany) according to the manufacturer's protocol. The primer pair: F: 5′-CTGGAGACCACTCCCATCCTTTCT-3′ and R: 5′-GATGTGGCCATCACATTCGTCAGAT-3′ was used to amplify and genotype the participants, using PCR and agarose gel electrophoresis. PCR was performed at 95˚ pre-denaturation for 4 min, followed by 33 cycles of denaturation at 94˚ for 45 s, annealing at 60˚ for 30 s, extension at 72˚ for 40 s, and one final extension cycle of 72˚ for 5 min.

The participants exercised on a treadmill according to the Bruce protocol; the VO2 max values were estimated by algorithms incorporated into the stress test software. Anthropometric measures were calculated using body composition analyzer (Inbody 220, South Korea). Flexibility was examined by the Wells Sit and Reach test. Two-minute push-up and sit-up tests were used to determine muscle endurance. The maximal and average strength of hand mascules were measured by mechanical dynamometer. Arterial evaluation was performed by SphygmoCor (Atcore Medical, Sydney, Australia), and included assessments of the augmentation index, central blood pressure, aortic pulse pressure, reflection time of the propagated pulse as well as ejection duration, and subendocardial viability index. Resting heart rate was determined by the participants measuring their rate, following instruction by the researchers, for three mornings before getting up from bed. Blood pressure was measured before and after the exercise protocol using an automated brachial oscillometric device (M10-IT; Omron, Kyoto, Japan). Pulse pressure amplification was defined as the increment in pulse pressure from the aortic to radial regions. Circulatory power was calculated as the product of oxygen and maximal systolic Blood Pressure (SBP) during exercise, and the rate-pressure product was calculated as the maximal HR multiplied by maximal SBP per 100 means divided by 100. HR reserve (HRR) was calculated using the formula below: HRR = [(peak HR –HR at rest) / (220–age–HR at rest)] × 100

The genotype and allele frequencies were determined by gene counting. The data analysis was performed using IBM-SPSS Version 24 for comparisons between the genotypes and determinant factors for physical performance. The Mann–Whitney U-test and ANOVA statistical analyses were carried out to compare the physical performance as well as the hemodynamic indices in different ACE genotype groups. Multivariate analysis modeled the determinant factors for ventricular function, arterial function, and physical performance. The significant level was set at 0.05.

Results

In order to find out the frequency of ACE I/D genotypes, PCR and gel electrophoresis techniques were performed in our 146 samples (table 1). The frequencies of DD, ID and II genotypes were 38.5%, 41.5%, and 20%, respectively (figure 1). The PCR products for different genotypes are shown in figure 1.

Table 1. Genotypes and allele frequencies of ACE I/D gene in the total study sample (N=146)

	Genotype frequencies (%)			Allele frequencies (%)	
	DD	ID	II	D	I
N=146	56 (38.5)	61 (41.5)	29 (20)	173 (59.3)	119 (40.7)

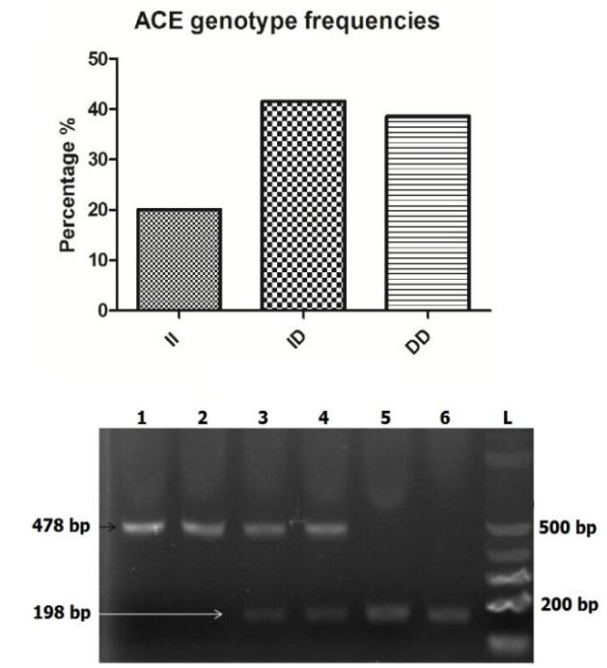

Figure 1. *ACE* I/D gene polymorphism genotyping. The investigation of *ACE* genotype frequencies in the population showed higher percentages for ID genotypes. Different genotypes of *ACE* I/D polymorphism are shown on 1.5% agarose gel. Genotype II (wells 1 and 2), genotype ID (wells 3 and 4), and genotype DD (wells 5 and 6), along with 100bp DNA ladder (L)

Of the 43 people who participated in physical performance tests, there were 26 people with allele I (ID and II) and 17 people without allele I (DD). The statistical data analysis using the Mann–Whitney U-test showed no associations between ACE polymorphisms and physical performance indices. Nevertheless, it showed an association between cardiovascular factors such as pulse pressure (PP) amplification (P=0.02), heart rate increase during exercise (P=0.037), and heart rate at rest (P=0.05) with the ACE polymorphism (Table 2). In addition, the association between PP amplification and the polymorphism was significant even after controlling for age (P=0.004). In multivariate analysis for exercise endurance, the ACE polymorphism effect was unmasked (β=0.34, P=0.047) after controlling for age, arterial stiffness, diastolic function, heart rate, and body fat (R2=53.8%). More importantly, the ACE polymorphism was a significant determinant of ventricular performance as expressed by PP amplification (P=0.004) after adjusting for age. Similarly, rate pressure product was predicted by the ACE polymorphism (P=0.049) and arterial stiffness (P=0.015) even after adjustment for age (table 3).

Table 2. The measures between physical performance and cardiovascular factors with two ACE genotype groups (DI/II and DD)

	DI and II variants (%)	DD variant	P.V
Tolerated distance	0.74 (0.41)	0.76 (0.11)	0.67
Tolerated time	9.5 (1.3)	9.5 (1.0)	0.99
VO_2 Max	35.2 (7.3)	35.7 (6.7)	0.84
Max MET	12.6 (1.2)	12.6 (1.3)	0.78
Sit and Reach	45.9 (8.3)	44.0 (6.5)	0.53
Maximal force	81.1 (13.7)	79.6 (14.9)	0.89
Average force	54.6 (10.3)	52.2 (14.9)	0.60
Resting HR	80.9 (13.4)	90.4 (12.6)	0.05
Resting radial SBP	126.4 (11.3)	132.6 (10.7)	0.07
Resting DBP	81.6 (10.6)	78.8 (15.6)	0.84
Radial pulse pressure	38.9 (8.9)	40.3 (8.7)	0.37
Aortic SBP	98.7 (10.5)	99.4 (10.7)	0.99
Aortic DBP	75.1 (8.5)	75.9 (9.3)	0.81
Aortic pulse pressure	23.6 (5.0)	23.5 (5.2)	0.74
PP amplification	164.0 (11.2)	172.7 (10.4)*	0.02
SBP	55.1 (15.7)	60.5 (15.8)	0.39
HR	99.8 (16.6)	90.9 (11.2)*	0.03
Circulatory power	6741.3 (1695.1)	7034.9 (1538.5)	0.77
Rate-pressure product	347.1 (45.1)	357.9 (23.7)	0.63
Heart rate reserve	0.99 (0.1)	1.0 (0.05)	0.48
Adjusted augmentation index	4.9 (8.0)	4.7 (7.9)	0.98
Pulse wave reflection time	155.0 (12.5)	149.2 (6.8)	0.12

*pulse pressure (PP) amplification
*HR: heart rate

Table 3. Multivariate analysis for the rate-pressure product

Source	Type III Sum of Squares	df	Mean Square	F	P.V
Corrected Model	22411.27(a)	7	3201.61	2.88	0.02
Intercept	124846.00	1	124846.00	112.38	0.00
Age	2759.20	1	2759.20	2.48	0.13
ACE polymorphism	4679.31	1	4679.31	4.21	0.05
Level of adjusted augmentation index	13821.24	3	4607.08	4.15	0.01
Interaction	2736.78	2	1368.39	1.23	0.31
Error	32215.63	29	1110.88		
Total	4610291.42	37			
Corrected Total	54626.90	36			

Discussion

Physical performance, like other human phenotypes, results from an interaction between genetic factors and environmental stimuli. These athletic phenotypes, like endurance capacity, muscle performance, and psychological aptitudes have been mentioned to have genetic influences.[2,9] There is a complicated relationship between physical performance with a physical activity, environmental factors, and genetic factors, and these associations differ between individuals and populations. Therefore, finding and selecting biological markers to achieve a reliable measure for the evaluation of physical performance can be challenging. Many studies support a significant association for increased endurance-based physical performance with the ACE II genotype compared to D-allele carriers (DD+ID).[8,10-12] On the other hand, there are also some other studies that contradict these findings and suggest an association of D allele/DD genotype with endurance athletes' phenotypes.[13-15] However, there have been some studies that suggest no association between the ACE I/D polymorphism and physical performance.[16,17] In a meta-analysis by Ma et al., the association of sport performance with ACE I/D polymorphisms in 25 previous studies were summarized.[18] While the meta-analysis showed no statistically significant association between the ACE I allele and endurance-based activities, it suggests a significant association between the ACE II genotype and endurance-oriented athletic activities. These results demonstrate complicated mechanisms of physical performance containing genetic bases; many genes may influence the development of physical fitness. For instance, it has been noted that the influence of the ACE I/D gene polymorphism might be due to variations in nearby loci such as growth hormone (GH) gene that are linked to the ACE genotype.[1]

In our study, we did not find any association between the ACE gene I/D polymorphism and physical performance in univariate analysis, which might be due to the homogeneity of the sample; however, we found an association between the ACE polymorphism and cardiovascular markers. In addition, multivariate models of analysis showed this polymorphism had an important role in the maximum tolerated distance of running among participants. In the DD group, the vascular function and cardiac performance were more effective, and it seems DD genotypes had a better response to exercise. In other words, it appears that in such a normal and healthy population, the DD genotype holders have more renin-angiotensin activity, which in turn causes a better hemodynamic response in the cardiovascular system. The increase in the renin-angiotensin system in abnormal ranges or in an individual lacking cardiovascular compensation could lead to an increased cardiac load, and finally cardiac hypertrophy, ventricular stiffness, and diastolic dysfunction. This process could finally lead to reduced physical performance. Therefore, the impact of the ACE polymorphism is not as simple as it was previously presumed. It may depend on the level of cardiac reserve and

compensation. As a result, the DD genotype leads to an increased risk of high altitude syndrome in climbers and ischemic heart disease in elderly. All of these could be explained by the role of ACE.

The distributions of three ACE genotypes in our study were 38.5%, 41.5% and 20% for DD, ID, and II, respectively. Our findings are in agreement with previous studies from Iran, in which the I and D allele frequencies were similar.[19-21] Our estimated ACE different genotype frequencies are significantly different from those reported for Korean and Japanese populations, which, in comparison to our results, show higher D allele frequencies and lower I allele frequencies. Furthermore, the I/D allelic frequencies are different from those reported in European countries, and the I allele frequency is lower in our population. However, our frequencies were similar to the rates recorded in the Greek population.[22]

The limited sample size for the second phase of the study could be a major limitation and a source of type 2 error. Further investigation with larger sample sizes could be useful, particularly in terms of ascertaining cardiovascular variables that showed non-significant trends between the groups. Our findings on the frequencies of ACE I/D polymorphisms could be generalized to the healthy Iranian male population. The results of the second phase of this study could be generalized to the similar groups; however, the purpose to explore the pathophysiological relations between the elements. ACE polymorphisms are more likely to correlate with cardiovascular determinants of physical performance rather than musculoskeletal factors. The net impact of ACE polymorphism is likely to be on the cardiovascular system rather than musculoskeletal condition. Therefore, by ACE activation young males (with good cardiovascular reserve) would take benefits whereas it may followed by adverse performance when a decompensated cardiovascular state is present.

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Conflict of Interest

The authors declared that they have no conflict of interest.

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