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Investigation of the Effects Of *ACE* Gene Polymorphism on Athletic Performance in Active Adult Males

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Abstract

Studying genes associated with exercise-related physiological systems and metabolic pathways has proven to be an effective method for identifying genetic markers associated with enhanced athletic performance. One of the extensively studied genes in genetics is "Angiotensin I-converting enzyme (*ACE*)" which has a significant role in regulating blood pressure, plasma volume, and the development of cardiac and skeletal muscle. The objective of this study was to determine the different results and genetic variations in the effects of *ACE* gene polymorphisms on the performance of the 30m sprint test and the arrowhead agility drill test in active adult males after a 6-week training program. Following the manufacturer's instructions, genomic DNA was extracted from participant mouth swab samples using the Buccalyse DNA Extraction Kit from Isohelix. The Statistical Package for the Social Sciences (SPSS) 25 application was used to perform statistical analysis on the research data. The findings indicate that 45.7% of the participants possess the I/D genotype, 31.4% possess the I/I genotype, and 22.9% possess the D/D genotype. There was no notable disparity in the performance of the arrowhead agility test and the 30 m sprint test among the different genotype groups ($p < 0.05$). Our analysis revealed a lack of association between genetic variants and the values obtained from performance measurements in the test outcomes. Due to the involvement of various genes and variables, further research is needed to improve the predictability of approaches to *ACE* gene variations, considering their performance-enhancing benefits.

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Introduction

Following the conclusion of the Human Genome Project (IGP; 1990-2003), research into the association between genetic variants and athletic ability has become increasingly important (Ahmetov and Fedotovskaya, 2015). The objective of genetics studies in sport is to identify specific genes that have an impact on athletic performance, investigate their genotype-phenotype relation, and evaluate inherent athletic abilities (Kaman et al., 2017). Controversial findings exist, but the mechanism of genetic impact on athletic performance still needs to be clarified. The genetic findings applied in sport research, show that individuals can achieve high levels of athletic performance within their genetic limitations. The integration of genetic traits into talent identification systems is crucial for the development of athletes' training programs, assessment of their utility, prediction of optimal performance, and identification of critical strengths and weaknesses (Bulgay and Zorba, 2022).

Individual differences in physical fitness may be linked to genetic inheritance, traits uncovered via individual research, and the relationship between epigenetics and the shared environment. (Sahin, 2005). A well-recognized and effective approach to identify genes linked to athletic performance is by prioritizing genes involved with exercise-related physiological systems and metabolic pathways. In this regard, Angiotensin I-converting enzyme (*ACE*) is a critical component of the Renin-angiotensin system (RAS) in the development of athletic performance. (Fatini et al., 2000). RAS is identified by its ability to regulate plasma volume and blood pressure, as well as influence on cardiac and skeletal muscle growth.

The *ACE* gene variants (II, ID, and DD) have distinct effects on athletic performance. Individuals with elevated *ACE* enzyme levels experience a decline in their long-term aerobic endurance performance. This is because the increased blood flow caused by the higher enzyme levels is unable to adequately supply the muscle tissue with sufficient blood flow.

On the other hand, individuals with low *ACE* enzyme activity exhibit high levels of aerobic endurance performance and exercise efficiency. This is attributed to an abundance of blood in circulation (Cerit et al., 2006). Individuals possessing the D variant of the *ACE* gene express higher levels of angiotensin II and plasma *ACE* enzyme activity, which has been associated with enhanced strength and power production during workouts (Fatini et al., 2000). In this context, the objective of this study was to determine the different results and genetic variations in the effects of *ACE* gene polymorphisms on the performance of the 30m sprint test and the arrowhead agility drill test in active adult males after a 6-week training program.

The findings of our research are expected to assist individuals to sports disciplines that take their genetic profiles into consideration and build training regimens that take individual traits into account.

Material and methods

Ethical approval

The research was performed in compliance with the Declaration of Helsinki, and approval was secured from the Lokman Hekim University Non-Interventional Clinical Research Ethics Committee, decision number 2023-246/1.

Study population and sample collection

The research group was founded on a volunteer basis with 39 male students aged 19 to 24 who study taekwondo at Lokman Hekim University, Faculty of Sports Sciences.

Data collection tools

The students who volunteered to participate in the study were provided with details and signed the "Informed Volunteer Consent Form". Before initial examination, participants submitted mouth swab samples for DNA analysis. The arrowhead agility test and the 30-meter sprint test were administered to volunteers whose measurements were taken at the beginning and end of 6 weeks (pre-test/post-test) to assess their anaerobic performance levels.

Arrowhead agility drill test

The Arrowhead agility test assesses athletes' agility, combustibility, and change of direction abilities. This test was initially designed to assess soccer players' physical abilities, however it has since been used to other sports that demand agility. The test assesses a participant's speed, power during impact, control of the body, and ability to rotate in various directions. The arrowhead agility test evaluates athletes' agility, exploding ability and capacity to change direction. For the purpose of the test, a photocell and funnel are required. The athlete positions themselves behind a designated starting line (A) that is established as a component of the test and commences at their discretion. The athlete accelerates rapidly towards either the center cone (C) or (E), executes a sharp turn, and then sprints towards the far cone (D) before returning to

the initial position. The test records the athlete's completion time. In order for the test to be declared genuine, the athlete is required to perform the test a total of four times, with two repetitions on the right side and two on the left side (Lockie and Jalilvand, 2017).

30 meters sprint test

The participants' 30 m sprint test were measured using a Newtest, Finland brand photocell stopwatch device. The participants' running times for this distance were measured using a photocell stopwatch installed between 0 and 30 meters. The best of two attempts was measured in seconds. Prior to testing, participants performed customary warm-up exercises, which included general and particular exercises. Participants engaged in exercise three times per week to enhance their anaerobic and aerobic thresholds through long-term brisk runs, as well as improve muscle endurance through circuit training.

ACE rs4343 polymorphism analysis

The molecular testing for the Sport Science Faculty cadets were conducted at the Damagen Genetic Diagnosis Center in Ankara. Genomic DNA was extracted from oral swabs utilizing the Buccalyse DNA Extraction Kit (Isohelix, UK), in accordance with the manufacturer's guidelines. The DNA content was measured utilizing a Nano Drop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The genotyping of the single-nucleotide polymorphism (SNP) was conducted with the KASP sequencing kit (LGC Genomics is a Beverly, CA, USA). The KASP assay kit incorporates two allele-specific forward primers and one common reverse primer. The

main concern with the ACE gene is the SNP variant rs4343. The PCR reaction was executed employing the 7500 Real-time PCR System (Applied Biosystems, Foster City, CA, USA). Following that, fluorescence observations were carried out and the acquired data were evaluated in accordance with other studies (He et al., 2014).

Statistical analysis

The data was statistically analyzed using the Statistical Package for the Social Sciences (SPSS) 25 application. Participants' physical performance measurements were compared to data using one-way analysis of variance (ANOVA), Kruskal Wallis analysis of variance (with multiple comparison test), and multiple regression model to analyze effects and allele frequencies. The compliance of allele frequencies with Hardy-Weinberg equilibrium was investigated. $p < 0.05$ was considered statistically significant.

Results and Discussion

The study explored the impact of ACE gene rs4343 variants on the performance of the participating volunteers. The participants' performance was assessed using the arrowhead agility drill test and the 30-meter sprint test. These tests were conducted at the beginning and end of the 6-week training period. The ACE gene groups results were compared using an ANOVA

test, whereas the pre-test and post-test values within the ACE groups were compared using a dependent groups t-test. ACE genotype distribution is presented in Table 1.

Table 1 demonstrates that 45.7% of the individuals have the I/D genotype, 31.4% have the I/I genotype, and 22.9% have the D/D genotype. When 35 participants genotype, frequencies were evaluated, the I/D genotype was shown to be the most common one. Table 2 displays the results of the 30-meter sprint test. The average time for the pre-test was 5.58 seconds with a standard deviation of 0.54, while the average time for the post-test was 5.52 seconds with a standard deviation of 0.56. The mean duration for the right foot arrowhead agility test (pre-test) was 9.71 seconds with a standard deviation of 0.78, but the post-test mean was 9.76 seconds with a standard deviation of 0.74. The average time for the arrowhead agility test using the left foot (pre-test) was 9.71 seconds (± 0.83) initially and 9.67 seconds (± 0.79) at the conclusion. The arrowhead agility drill tests revealed no significant variation in results among different genotypes. The F and p values exceed the significance level for every test. The post-test data for the left foot showed a somewhat lower p value ($F=2.345$ and $p=0.112$) compared to the other tests, suggesting the presence of potential bias. Nevertheless, the disparity did not reach statistical significance (Table 3).

Table 1. Participants were distributed according to their ACE genotype

		n	%
ACE	D/I	16	45.7
	I/I	11	31.4
	D/D	8	22.9
	Total	35	100.0

Table 2. Results of participants' physical performance tests prior to and following the test

	n	min	max	mean	ss
30 m (pre-test)	35	4.72	6.77	5.58	0.54
30 m (post-test)	35	4.27	6.78	5.52	0.56
Arrowhead agility drill test (right foot-pre-test)	35	8.75	11.71	9.71	0.78
Arrowhead agility drill test (left foot-pre-test)	35	8.70	11.71	9.71	0.83
Arrowhead agility drill test (right foot-post-test)	35	8.71	11.25	9.76	0.74
Arrowhead agility drill test (left foot-post-test)	35	8.73	11.36	9.67	0.79

Table 3. Comparison of physical performance and anthropometric measurements on the basis of *ACE* genotypes of participants between groups

		n	mean	ss	F	p
30 m (pre-test)	D/I	16	5.44	0.47	0.971	0.390
	I/I	11	5.70	0.63		
	D/D	8	5.69	0.52		
30 m (post-test)	D/I	16	5.39	0.59	0.755	0.478
	I/I	11	5.61	0.56		
	D/D	8	5.64	0.51		
Arrowhead agility (right foot -pre-test)	D/I	16	9.60	0.59	0.479	0.624
	I/I	11	9.70	0.82		
	D/D	8	9.94	1.07		
Arrowhead agility (left foot-pre-test)	D/I	16	9.62	0.69	0.724	0.492
	I/I	11	9.61	0.86		
	D/D	8	10.02	1.07		
Arrowhead agility (right foot-post-test)	D/I	16	9.60	0.55	0.986	0.384
	I/I	11	9.76	0.77		
	D/D	8	10.06	1.01		
Arrowhead agility (left foot-post-test)	D/I	16	9.44	0.58	2.345	0.112
	I/I	11	9.66	0.78		
	D/D	8	10.16	1.04		
Height (cm)	D/I	16	1.77	0.06	0.569	0.571
	I/I	11	1.75	0.10		
	D/D	8	1.75	0.07		
Body weight (kg)	D/I	16	73.00	9.35	1.200	0.315
	I/I	11	68.73	11.91		
	D/D	8	65.25	16.17		

Individuals following a similar training routine may have various degrees of performance improvement.

In such kind of research, common variables such as sport discipline and status, gender, age, ethnicity, and geography should be taken into account in order to investigate the possible effects

of inherited differences (Cerit, 2022). Several studies (Alvarez et al., 2000; Gayagay et al., 1998; Nazarov et al., 2001) have examined the correlation between *ACE* genotype and aerobic endurance in athletes participating in swimming, rowing, cycling, handball, and running (at different distances).

These studies treated all participants as a single group, without considering any differences in sport or distance. Findings of these studies are inadequate to elucidate the resistance observed in relation to the requirements for the *ACE* genotype. Similarly, a study involving 404 participants from 19 different sports branches found that the frequencies of specific gene variations did not vary between athletes and the control group (Myerson et al., 1999). Additionally, there was no correlation observed between specific gene variations related to endurance performance in elite athletes (Rankinen et al., 2000; Sonna et al., 2001). The challenging outcomes of these heterogeneous study constrain the capacity to identify performance disparities among individuals resulting from genetic inheritance.

Unlike the inconsistent studies in question, a unique study conducted with comparable training loads, tailored to the specific characteristics of each sport, revealed that the frequencies of *ACE* gene polymorphism genotypes among elite basketball and volleyball players were similar. Furthermore, the gene variants between these two sports branches were also found to be alike. In addition, there was no notable disparity in the distribution of genotypes and alleles between top athletes and inactive athletes, as well as among different athlete subgroups. However, male volleyball players exhibited a higher occurrence of the DD genotype compared to basketball players (Suel and Pehlivan, 2015). Another study corroborated these findings by demonstrating that elite athletes possessing the *ACE* DD genotype had superior performance in sports that demanded anaerobic ability, such as short distance running, long jump, high jump, discus throwing, and short distance swimming (Ulucan et al., 2013). The findings from our investigation, as presented in

Table 1, indicate that 45.7% of the individuals (n = 35) possessed the I/D genotype, 31.4% had the I/I genotype, and 22.9% had the D/D genotype. Table 2 indicates that there is no statistically significant disparity between the pre-test and post-test values for the physical performance test evaluations of the participants. However, it has been shown that elite athletes with the II genotype can perform better than those with the DD genotype in sports requiring aerobic and muscular endurance, such as middle- and long-distance running, walking, and skiing (Holdys et al., 2011). Bulgay et al. (2020) conducted a similar study on *ACE* gene polymorphisms and elite athlete performance status, which found that certain gene variants, such as hereditary variables, had a beneficial effect on sports performance.

Eken et al. (2021) performed a study to investigate the association between *ACE* I and D polymorphisms and athletic performance. It was discovered that genetic characteristics can influence an individual's level of achievement in specific athletic disciplines. More specifically, researchers found soccer players who possessed the *ACE* DD genotype are more commonly found among those who succeed in sports that need high levels of speed and power. On the other hand, individuals with the *ACE* II genotype are more likely to be impacted by endurance sports. Nevertheless, our study (Table 3) did not reveal any statistically significant disparity in the inter-group comparisons of pre-test and post-test physical performance measures depending on the *ACE* genotypes on the individuals. Current research indicates that there is a direct correlation between the *ACE* D allele and the improvement of short-term aerobic endurance and anaerobic performance. On the other hand, the *ACE* I allele may be linked to a more favorable enhancement in medium and long-term aerobic

endurance performance when exposed to aerobic training (Cam et al., 2005). The *ACE* DD genotype is anticipated to exhibit a more favorable response to training in the enhancement of short-term aerobic endurance, while the *ACE* I gene exerts a significant impact on endurance performance during intense exertion lasting between 10 and 30 minutes. Furthermore, research has demonstrated that persons who possess the *ACE* DD genotype have greater muscle strength and anaerobic capacity improvements in response to training compared to individuals with the II genotype (Folland et al., 2000).

The study conducted by Cerit et al. (2006) yielded substantial advancements in understanding the relationship between *ACE* gene variation and performance. Specifically, research has indicated that individuals with the *ACE* DD genotype have a greater advantage in short-term aerobic performance improvement following aerobic endurance and muscular endurance training. Furthermore, there is a clear linear trend in performance improvement, with *ACE* DD genotype showing the highest improvement, followed by *ACE* ID genotype, and then *ACE* II genotype. The results of this study suggest that the *ACE* genotype may play a role in improving performance in different sports disciplines. Individuals with the *ACE* DD genotype is anticipated to exhibit superior performance, especially in brief-term activities that demand maximum exertion (Cerit et al., 2006). A subsequent study was undertaken on similar groups to investigate the correlation between the *ACE* gene and athletic performance. The study analyzed many factors such as short-term aerobic endurance, muscular endurance, body fat percentage, and body weight to assess the impact of exercise and candidate genes. The results

demonstrated statistically substantial disparities across the putative gene variants. Nevertheless, it has been determined that these variations are influenced by one's lifestyle and level of physical activity. Additionally, alterations in metabolism, environmental factors, and lifestyle choices have been found to have similar effects on candidate gene variants throughout time. Cerit (2018) and Yildirim et al. (2022) have reported that the *ACE* gene variant (DD) initially had a higher prevalence compared to the other gene variants (ID and II). Merely studying genetic factors alone is inadequate for the enhancement of physical performance (Beunen et al., 2010). Environmental factors, anatomical structure, alterations in the ratio of skeletal muscle fibers, lifestyle, and consistent training all play a crucial role. The future will see the development of polygenic profiling methods with improved classifications, as well as the introduction of genetic variants into these tools, which will increase the number of markers reflecting athletic ability.

As scientific research, technical breakthroughs, and genomic discoveries progress, genetic information may become more relevant in the realm of sports, although this may/mustnot always be the case when evaluating performance. While there might be significant variations in estimations driven by heredity, it is widely acknowledged that hereditary factors have effect on all human traits. (Polderman et al., 2015). Furthermore, the majority of traits exhibit complexity, indicating that they are impacted by multiple genes. Even qualities with a high degree of heritability are subject to the influence of numerous genetic variants and enviromental impact (Yengo et al., 2018). Insufficient sample size is a significant constraint in genetic investigations, which we have recognized as a drawback in our study (Varillas-

Delgado et al., 2022; Zilberman-Schapira et al., 2012). A substantial sample size is underscored as necessary to guarantee precise findings and uphold the statistical significance of disparities.

Conclusion

Our research focused on analyzing the potential impact of genetic differences on sports performance, specifically in relation to the outcomes of the 30-meter sprint test and arrowhead agility drill test. Our investigation found no statistically significant correlation between *ACE* gene variations and changes in performance. These data indicate that genetic variants do not impact the performance assessment values derived from these test results. Hence, it is deemed necessary to do further study in order to enhance the predictability of approaches to *ACE* gene variations. Given the circumstances, it is expected that the results of our research could potentially enhance other investigations in the area of sports genetics. Furthermore, we suggest that future studies prioritize longitudinal designs with substantial sample sizes and long-term homogeneous groups to thoroughly investigate the impact of candidate genes on athletic performance.

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None.

Authors' contributions

Ö. Coşkun:

Investigation; Resources; Roles/Writing- original draft and Writing.

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Conceptualization; Data curation; Formal analysis; Self-Funded; Investigation; Methodology; Project administration; Resources; Software; Validation; Visualization; Roles/ Writing original draft; and Writing -review & editing.

M. Anılır:

Resources; Supervision; Validation; Visualization; Roles/Writing -original draft; and Writing -review & editing.

M. Cerit:

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Conflict of interest

Authors declare no conflict of interest.

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