

## EXAMINATION OF THE RELATIONSHIP BETWEEN ANGIOTENSION CONVERTING ENZYME (ACE) rs4343 GENE POLYMORPHISM AND YO-YO INTERMITTENT RECOVERY TEST (LEVEL 2) PERFORMANCE IN NON-ELITE ATHLETES

DOI: <https://doi.org/10.46733/PESH25141123d>

(Original scientific paper)

Hakan Dakilir<sup>1</sup>, Mesut Cerit<sup>2</sup>, Didem Çatalo<sup>3</sup>, Murat Anilir<sup>4</sup>, Metin Dalip<sup>5</sup>

<sup>1</sup>Sports Science Faculty, Lokman Hekim University, Ankara 06510, Türkiye

<sup>2</sup>Sports Science Faculty, Lokman Hekim University, Ankara 06510, Türkiye

<sup>3</sup>Sports Science Faculty, Kırıkkale University, Kırıkkale 71460, Türkiye

<sup>4</sup>Sports Science Faculty, Lokman Hekim University, Ankara 06510, Türkiye

<sup>5</sup>Faculty of Physical Culture and Health, University in Tetova, 1200 Tetova, Macedonia

### Abstract

The ACE gene, extensively studied in the context of athletic performance development, has shown a substantial influence on physical performance. The ACE gene encodes the angiotensin-converting enzyme, which has a vital function in regulating blood pressure by controlling the amounts of body fluids. Elevated levels of ACE enzyme activity result in vasoconstriction, impeding the delivery of oxygen and nutrients to cells, thereby diminishing endurance during prolonged periods of physical effort. The aim of this study was to evaluate the potential influence of ACE gene polymorphism on the performance of non-elite male athletes in the Yo-Yo Intermittent Recovery Test (Level 2). A total of 53 male athletes who were not considered to be elite took part in this study. Among them were 30 basketball players and 23 volleyball players. We performed the Yo-Yo Intermittent Recovery Test (Level 2) at both the beginning and the end of the 6-week study to evaluate the levels of anaerobic and aerobic power, as well as recovery. The genotyping technique employed either the KASP genotyping approach or microarray analysis. We employed the Independent and Dependent Groups T tests to evaluate differences between the groups and measure changes within the group over a specific time period. Furthermore, the Chi Square Test was employed to assess the disparities in ACE genotype frequencies across the populations. The ACE genotype distributions of the entire cohort were assessed, and the disparities between the basketball and volleyball branches were analyzed. The basketball team exhibited a genotype distribution of 46.7% for ID, 33.3% for DD, and 20.0% for II. The volleyball group had a genotype distribution of 47.8% for ID, 39.1% for DD, and 13.0% for II. There was no significant difference ( $p > 0.05$ ) in the genotype frequencies between the two groups. This study found that non-elite male athletes with ACE ID and DD genotypes showed better performance improvement compared to those with II genotypes during short-term and intermittent high-intensity maximum efforts. Additionally, the ACE gene polymorphisms exhibited a linear distribution, with  $ID \geq DD > II$ .

**Key words:** ACE, Yo-Yo intermittent recovery test (Level 2), anaerobic performance.

### Introduction

Several genetic factors, including body structure, muscle fiber proportions, the ability of the circulatory and respiratory systems, metabolic functioning, and athlete psychology, have a big impact on how well an athlete can build, maintain, and improve their performance (Ulucan et al., 2016; Ahmetov & Fedotovskaya, 2015). Although athletes can improve their athletic performance through devotion and a well-designed training program, some individuals may naturally exhibit remarkable levels of athletic performance without the need for intensive training methods (Tucker & Collins, 2012). Various research in the field of sports genetics has shown a significant association between genetic variables and athletic ability (McAuley et al., 2021). Recent meta-analyses of twin and family studies have determined that genetic factors contribute to around 21% to 48% and 19% to 54% of the observed variation (De Geus et al., 2014; Lightfoot et al., 2018). According to De Moor et al. (2007), genetic factors account for almost 66% of the variability in athlete status. Training methods, diet, ergogenic supports, geography, medical and social support opportunities,

and environmental factors can all contribute to the remaining disparities (Beck et al. 2015; Bezuglov et al. 2023). The exceptional performance of Olympic athletes is attributed to the precise interplay of factors such as fortuitous pairings in genetic sequences, optimal surroundings, and meticulous planning, execution, and supervision of training (Hughes et al., 2011; Williams & Folland, 2008).

Ahmetov et al. (2024) reported that their study identified 253 genetic markers with a minimum of 149 variations associated with various physical activity traits. The findings of this research indicate that individuals have the capacity to enhance their biomotor abilities, such as speed, strength, and endurance, up to their genetic potential (Ahmetov et al. 2024). Among the genes associated with performance, the *ACE* gene stands out due to its extensive exploration in the development of athletic performance and its significant impact on physical performance (Cerit et al., 2023). The *ACE* gene encodes angiotensin-converting enzyme, which plays a crucial role in blood pressure regulation through the management of bodily fluid levels. ACE is found in large amounts on the outer layer of vascular endothelial cells. It controls the amount of Ang II by changing naturally occurring Ang I and bradykinin. While ACE levels in plasma are rather resistant to influences from environmental, humoral, and metabolic variables, there are significant variations across individuals (Gao, 2006). Increased levels of ACE activity cause vasoconstriction, which hinders the transportation of oxygen and nutrients to cells, leading to reduced endurance during lengthy periods of physical exertion. However, activities that require rapid and forceful exertion, like brief but intense sprints lasting 8–12 seconds, show enhancements. People with increased ACE activity had lower MaxVO<sub>2</sub> and restricted improvement in endurance performance because of increased vasoconstriction, which restricts adequate blood supply to the muscles. According to reports, athletes with reduced ACE activity see notable improvements in their endurance capacity as a result of the heightened delivery of oxygen and nutrients to the tissues (Cerit et al., 2006).

The *ACE* gene not only regulates blood pressure but also impacts skeletal muscle functioning. The enzyme ACE helps angiotensin I change into angiotensin II. Angiotensin II is a stronger vasoconstrictor and also helps muscles grow in cases of overload-induced muscular hypertrophy (Woods & Montgomery, 2001; Cerit et al., 2006). The *ACE* gene exhibits two distinct variants. Their DNA possesses the I allele, characterized by the presence of an additional base pair, and the D allele, characterized by the absence of a base pair. There are three genotypes present in this polymorphism: DD, II, and ID. These disparities suggest that ACE plays a significant role in modulating the synthesis of Angiotensin II at the cellular level. Persons with the DD genotype exhibited the greatest levels of ACE in both serum and tissue, whereas persons with the II genotype had the lowest ACE levels (Rankinen et al., 2000; Zhao et al., 2003). Research (Myerson et al., 1999; Myerson et al., 2001; Cerit et al., 2006) has shown that individuals with a high frequency of the *ACE* D allele exhibit superior anaerobic performance, have a bigger increase in lean body mass during training, and demonstrate enhanced muscular strength growth as a result of resistance training. Some studies have revealed no evidence of a positive correlation between DD alleles and achievement in elite sprint/power sports (Eynon et al., 2012).

Identifying an athlete with the optimal genetic structure and successfully achieving the goal by considering several factors such as personalized training, lifestyle, environmental influences, and social connections is a challenging and intricate task (Cerit, 2018). Given a variety of circumstances, the interactions between candidate genes and their impact on training adaptation are complex and vulnerable to interpretation. The extensive and prolonged training regimens undertaken by professional athletes over numerous years may not suffice to secure a position on the podium. Each person's genetic potential and capabilities vary, therefore, it is quite likely that there will be individuals who exhibit varied responses to the same training. Despite individuals performing identical workouts simultaneously, their progress will vary with time. (Ilgun et al., 2020). The complex interaction between genetic and environmental factors greatly limits the capacity to determine athletic skills and create an accurate genetic plan for certain accomplishments. Individuals with identical genetic profiles exhibit more similar responses to training stimuli compared to individuals with distinct genetic profiles. Recent studies indicate that specific genetic factors have a significant influence on performance-related traits in response to both untrained and regular exercise. The athlete's initial performance status plays a critical role in determining the magnitude of performance enhancement achieved through training. The most prominent indication of the impact of lifestyle and environmental factors is the observation that training responses display individual variability.

The objective of our study is to examine the potential influence of *ACE* gene polymorphism on the performance of non-elite male basketball and volleyball players in the YO-YO Intermittent Recovery Test (Level 2). The findings of this study are expected to provide guidance for individuals in selecting sports

disciplines that align with their genetic profiles, optimize the performance of elite athletes, design training programs that take into account individual characteristics, and enhance our comprehension of the importance of genetic factors.

## **Material and Methods**

### *Participants*

The study sample comprised 61 male athletes aged 19-24 who were in excellent health and not part of the elite category. They participated in the research on a voluntary basis. The study's case group consisted of 30 young players in the basketball branch, while the control group contained 23 volleyball athletes. Prior to the study, the athletes signed written informed permission forms that included comprehensive information such as the study protocol and findings. Subsequently, an oral DNA sample was obtained from each subject using a cotton swab for genetic analysis. The study protocol (2023/250/1) was approved by the Ethics Committee of Lokman Hekim University, and the study procedure adhered to the principles outlined in the Helsinki (II) Declaration. All subjects went through to a six-week exercise program.

### *Exercise Program*

The research groups performed conditioning workouts to enhance both anaerobic and aerobic thresholds by engaging in long-duration steady-state runs. In addition, they prioritized enhancing muscle endurance with circuit training exercises. The program consists of one microcycle per week, three training sessions per microcycle, and one exercise session (equivalent to one unit lasting 90 minutes) per day, specifically in the evening. This results in a total duration of around 270 minutes per week.

### *Procedures*

The diligent and supportive researchers carried out the data collection and analysis phases. Task allocation was implemented to decrease measurement mistakes during the process of measurement. The application was meticulously developed in accordance with the athletes' training calendars, following a scheduled and organized approach. Prior to the measurement, participants were provided with information regarding the technique and details of the study one week in advance. The subjects' physical performance qualities were assessed using the Yo-Yo IRT 2 test before and after the training session. This test was conducted as a research study to examine aerobic capacity, anaerobic performance, and recovery rates. Before the study, all participants were already familiar with the testing methods due to the training and performance assessment processes carried out by their individual teams. The pre-test and post-test were completed using identical test protocols. Before the tests, the athletes engaged in traditional warm-up routines, which consisted of general activities such as 5-10 minutes of moderate jogging, followed by 5 minutes of active stretching of the upper and lower limbs. After the warm-up, players were given a three to five minute period of rest before performing the tests.

### *Data Collections*

The Yo-Yo IRT 2 test requires subjects to perform two 20-meter shuttle runs, guided by audio signals from a compact disc player, within a designated time frame of 5 to 15 minutes. The shuttle runs are conducted at progressively faster rates, with 10 seconds of active recovery time between each run. The Yo-Yo IRT 2 test has a duration of around 3, 5, 10, and 12 minutes. This test assesses the anaerobic energy utilization and recovery efficiency of elite athletes during rest periods between intense exercise repetitions. (Mayhew et al., 1985; Atkins et al., 2006). The outcome of the test is determined by the overall distance achieved. The individual being tested continues in running until they reach their limit and can no longer maintain their present speed. The Yo-Yo IRT 2 test evaluates the anaerobic capacity and the rate at which a trained individual recovers from high-intensity running. The test in question is extensively utilized in many individual and team sports to quantitatively assess athletic performance (Bangsbo et al., 2008).

### *Genotyping*

The genetic analysis of the oral swab samples obtained from the participants was carried out in collaboration with "Damagen Medical Genetics Laboratory (Damagen Genetic Diagnosis Center, Aziziye Mah. Cinnah Cad. No. 102/1/Ankara). Epithelial cell samples were obtained from all participants through the process of signing prospective forms. The study procedures were carried out in compliance with the principles outlined in the Declaration of Helsinki II. The Buccalyse DNA Extraction Kit from Isohelix was

used to isolate genomic DNA from the swab samples, following the manufacturer's stated technique. The concentration of DNA was evaluated using a NanoDrop spectrophotometer from Thermo Fisher Scientific, located in the USA. The study utilized the KASP genotyping method (LGC Genomics) to assess single nucleotide polymorphism (SNP) genotyping. This genotyping method utilizes competitive allele-specific PCR to accurately determine the presence of specific single nucleotide polymorphisms (SNPs) and insertions and deletions (Indels) at targeted locations, allowing for the identification of two possible alleles. The primer for the *ACE* gene SNP rs4343 was developed.

The KASP assay mix comprises three assay-specific unlabeled oligonucleotides, two allele-specific forward primers, and a common reverse primer (LGC Genomics). The KASP Master mix (2X) was acquired in a pre-made form, which includes the universal fluorescent dyes FAMTM and VICTM, as well as the Rhodamine X (ROX) dye as a positive reference. The DNA samples were treated with a mixture of SNP-specific KASP Primers and universal KASP Master mixture. The PCR reaction was then carried out using the 7500 Real time PCR System from Applied Biosystems. The resulting fluorescence readings were evaluated to acquire the data (He et al., 2014).

### Statistical analyses

This section of the study focuses on the findings derived by comparing the measures taken from the research participants and doing thorough reviews of these results. The researchers collected demographic information from the subjects and also investigated the variations in *ACE* gene polymorphisms. The Independent Groups T Test was utilized to assess disparities between the groups. This test was conducted to assess the statistical significance of the disparities between the mean values of performance metrics and physical attributes in the basketball and volleyball groups. The Dependent Groups T Test was utilized to assess the alteration inside the group over a period of time. This test assessed the statistical significance of the disparities between the beginning and posttest results to examine the impact of time on performance indicators within each sport branch group. In addition, the Chi Square Test was used to assess the distribution of *ACE* genotype frequencies among the different groups. The data analysis was conducted using SPSS, and a significance threshold of  $p < 0.05$  was deemed acceptable for all statistical tests.

### Results

The tables below present the data obtained from a comparative study of measures conducted on non-elite athletes participating in the research, along with thorough appraisals of these results.

Table 1. An analysis of *ACE* genotype frequencies in basketball and volleyball groups.

	ID	Group				Chi Square Test	
		Basketball		Volleyball		X <sup>2</sup>	p
		N	%	n	%		
<i>ACE</i>	ID	14	46,7	11	47,8		
	DD	10	33,3	9	39,1	0,497	0,780
	II	6	20,0	3	13,0		

Table 2. An Independent Groups T Test was undertaken to analyze the physical and performance measures of basketball and volleyball groups.

	Grup				Independent Groups	
	Basketball		Volleyball		T Test	
	X	ss	X	ss	t	p
<b>Pre-test (Yo-Yo distance 1)</b>	439,33	189,41	638,26	158,16	-4,064	0,000
<b>Post-test (Yo-Yo distance 2)</b>	553,33	231,77	681,74	142,44	-2,484	0,016
<b>Height</b>	1,76	0,08	24,85	60,79	-1,822	0,082
<b>Weight</b>	69,97	11,79	79,00	12,08	-2,736	0,009

Using the Chi Square Test, the study assessed whether there were differences in the frequencies of *ACE* gene polymorphism between basketball and volleyball groups. Within the basketball group, the prevalence

of participants with the ID genotype was 46.7% (n = 14), the DD genotype was 33.3% (n = 10), and the II genotype was 20.0% (n=6). The volleyball group had an ID detection rate of 47.8% (n = 11), a DD detection rate of 39.1% (n = 9), and an II detection rate of 13.0% (n = 3). Upon comparing the genotype frequencies between the two groups, the Chi Square value was determined to be 0.497, with a corresponding p-value of 0.780. These data indicate that there is no statistically significant disparity in genotype frequencies ( $p > 0.05$ ). Thus, there was no notable disparity observed in the distribution of *ACE* genotypes among persons participating in basketball and volleyball.

Table 2 assesses the statistical importance of the average disparities between the two groups. The performance and physical attributes of the participants in the basketball and volleyball groups were compared using the Independent Groups T Test. The Yo-Yo IRT 2 test (Pre Test) revealed that the basketball group had an average distance of 439.33 meters (sd = 189.41), whereas the volleyball group had an average distance of 638.26 meters (sd = 158.16). The measure's t value is -4.064, and the p value is 0.000, showing a significant difference between the two groups ( $p < 0.001$ ). In the latest test, the t value was determined to be -2.484 and the p value was 0.016, suggesting that there is a substantial ongoing difference between the groups ( $p < 0.05$ ). Furthermore, in relation to physical attributes, there was a significant disparity in average height ( $t = -1.822, p = 0.082$ ) and average weight ( $t = -2.736, p = 0.009$ ). Given that the p-value for height was just above .05, it may be concluded that this difference is marginally significant. Given that the p value for weight was below .05, it may be inferred that this disparity is statistically significant.

Table 3. Performance in basketball and volleyball groups changes with time.

		X	ss	Dependent Groups T Test	
				t	p
Basketball	Pre-test (Yo-Yo IRT 2)	439,33	189,41	-4,092	0,000
	Post-test (Yo-Yo IRT 2)	553,33	231,77		
Volleyball	Pre-test (Yo-Yo IRT 2)	638,26	158,16	-5,234	0,000
	Post-test (Yo-Yo IRT 2)	681,74	142,44		

Table 3 presents the findings of the Dependent Groups T Test, which assesses the changes in performance over time in the basketball and volleyball groups. The study evaluates the statistical significance of the performance variations between the initial and final tests within the same group. Within the basketball group, the mean distance covered in the Yo-Yo IRT 2 test was 439.33 meters (standard deviation = 189.41) initially, and 553.33 meters (standard deviation = 231.77) in the final test. The observed change is statistically significant ( $t = -4.092, p < 0.001$ ), suggesting a notable enhancement in the anaerobic capacity of the basketball group. The Yo-Yo IRT 2 test results for the volleyball group showed that the average distance covered increased from 638.26 meters (sd = 158.16) at the beginning to 681.74 meters (sd = 142.44) in the post-test. This suggests a substantial improvement in performance ( $t = -5.234, p < 0.001$ ).

Table 4. Comparative analysis of *ACE* gene polymorphisms in basketball and volleyball groups.

			Pre-test		Post-test		Wilcoxon test	
			X	ss	X	ss	Z	p
Basketball	ID	Yo-Yo IRT2	510,00	178,15	640,00	258,40	<b>-2,552</b>	<b>0,011</b>
	DD	Yo-Yo IRT2	338,00	121,64	452,00	189,08	<b>-2,325</b>	<b>0,020</b>
	II	Yo-Yo IRT2	443,33	253,75	520,00	175,27	<b>-1,807</b>	<b>0,071</b>
Volleyball	ID	Yo-Yo IRT2	654,55	153,65	680,00	144,50	<b>-2,215</b>	<b>0,027</b>
	DD	Yo-Yo IRT2	662,22	147,46	717,78	132,83	<b>-2,565</b>	<b>0,010</b>
	II	Yo-Yo IRT2	506,67	200,33	580,00	163,71	<b>-1,604</b>	<b>0,109</b>

Table 4 displays the outcomes of the Wilcoxon signed-rank test conducted to examine the changes in performance before and after the Yo-Yo IRT2 tests among various *ACE* (ID, DD, II) groups in the basketball and volleyball groups. This analysis assesses the statistical significance of the differences in performance between pre- and post-tests within each group. The average distance covered by individuals

with ID genotypes in the basketball group before the test was 510.00 meters (with a standard deviation of 178.15), but the average distance after the test was found to be 640.00 meters (with a standard deviation of 258.40). The observed change in the anaerobic capacity of the basketball group is statistically significant ( $Z = -2.552$ ,  $p = 0.011$ ), suggesting a notable improvement. The study found that individuals with the DD genotype had a mean distance of 338.00 meters (standard deviation = 121.64) before the test and 452.00 meters (standard deviation = 189.08) after the test. The shift in distance was statistically significant ( $Z = -2.325$ ,  $p = 0.020$ ). The average pre-test score for II genotypes was found to be 443.33 meters (standard deviation = 253.75), while the post-test score was 520.00 meters (standard deviation = 175.27). While the difference may not be immediately apparent, it is nonetheless worth mentioning ( $Z = -1.807$ ,  $p = 0.071$ ). The average distance in the ID subgroup of the volleyball group was measured to be 654.55 meters (with a standard deviation of 153.65) before the test and 680.00 meters (with a standard deviation of 144.50) after the test. This shift was determined to be statistically significant, with a Z-score of -2.215 and a p-value of 0.027. In the DD subgroup, the average distance covered in the pre-test was 662.22 meters with a standard deviation of 147.46. In the post-test, the average distance increased to 717.78 meters with a standard deviation of 132.83. The Wilcoxon test showed a significant change in distance, with a Z-score of -2.565 and a p-value of 0.010. In subgroup II, the initial distance of 506.67 meters (sd = 200.33) increased to 580.00 meters (sd = 163.71) in the post-test. However, this variation did not surpass the significance threshold ( $Z = -1.604$ ,  $p = 0.109$ ).

## Discussion

The study conducted by McAuley et al. (2021) has determined that the observed variations in performance can be ascribed to the existence of particular genetic variants. Researchers have conducted extensive research on the influence of *ACE* gene polymorphisms on athletic performance in endurance, power, and speed-based sports, revealing a significant association (Jeremic et al., 2019). Previous studies have repeatedly demonstrated a significant correlation between II genotypes (I alleles) and elite endurance athletes (Ahmetov & Fedotovskaya, 2015; Alvarez et al., 2000; Scanavini et al., 2002; Woods, 2009). On the other hand, researchers have found a substantial association between D alleles and athletes who rely on muscular strength. Specifically, research has established a connection between improved endurance performance and the I allele, which decreases the activity of the ACE enzyme (Pescatello et al., 2019). In contrast, Eider et al. (2013) have established a connection between the D allele and improved speed and power performance, attributing it to the higher quantity of fast-twitch muscle fibers. Studies have demonstrated that athletes possessing the ACE II genotype, characterized by reduced levels of the ACE enzyme, are capable of efficiently utilizing fatty acids as a source of energy during physical activity. Research has shown that this has been proven to improve performance throughout moderate (30 to 45 minutes) and prolonged physical activity. Long-distance runners who possess the genotype II and participate in the Olympic Games are thought to experience greater advantages compared to those with different genotypes in aerobic endurance activities of varying intensity levels (high-volume, medium, and low) and in high altitude environments. This is because to their higher ratio of capillary networks (Myerson et al., 1999; Pasha et al., 2001; Woods & Montgomery, 2001; Cerit et al., 2006; Shenoy et al., 2010; Valdivieso et al., 2017). In their study, Papadimitru et al. (2016) investigated the *ACE* I/D variations in a group of elite athletes that participated in the Olympics (346 athletes). They analyzed the athletes' performance in the 100, 200, and 400 meter finals, as well as their personal best sprint times. Their research revealed that none of the sprinters had *ACE* II genotypes, and endurance athletes did not have the *ACE* DD genotype (Papadimitriou et al., 2016). Myerson et al. analyzed the frequencies of *ACE* I/D and the genotype distributions in a study group of 495 athletes participating in 19 different Olympic sports disciplines. When examining a subgroup of 91 runners, they observed an increase in the frequency of the I allele as the distance increased (Myerson et al., 1999). Conversely, athletes with a high frequency of the D allele are believed to experience greater improvements in strength when participating in short-term, high-intensity anaerobic exercises (Myerson et al., 1999; Nazarov et al., 2001; Woods & Montgomery, 2001, Jones et al., 2002; Zilberman et al., 2012). They also have higher proportions of fast-twitch muscle fibers, which leads to increased muscle strength (Ahmetov et al., 2011; Erskine et al., 2014; Orysiak et al., 2014; Goddard et al., 2014; Goleva-Fjellet, 2016; According to these data, it has been concluded that *ACE* DD and ID polymorphisms offer more benefits in low-volume, high-intensity training regimens that include anaerobic characteristics (Ahmetov & Fedovskava, 2012; Sarmiento et al., 2018; Kelly & Williams, 2020). In our study, we found that the *ACE* genotype frequencies of non-elite male athletes did not show any significant

difference ( $p > 0.05$ ). However, similar to previous research, we also observed that there was no significant difference in the group of athletes participating in team sports such as basketball and volleyball, where the dominant energy system is anaerobic and fast-twitch muscle fibers are prevalent. There is a higher prevalence of ID and DD variations compared to II genotypes. In our study, we observed that 46.7% of the participants in the basketball group had the ID genotype ( $n = 14$ ), while 33.3% had the DD genotype ( $n = 10$ ), and 20% had the II genotype ( $n = 6$ ). In the volleyball group, 47.8% of the participants had the II genotype ( $n = 11$ ), 39.1% had the DD genotype ( $n = 9$ ), and 13% had the II genotype ( $n = 3$ ), (Table 1). A substantial statistical difference was seen in the performance disparities between the basketball and volleyball groups, irrespective of genetic variations (Table 2). However, there was a linear distribution in the performance differences observed before and after the Yo-Yo IRT 2 Test. In the basketball group, the distribution was ID > DD > II, while in the volleyball group, it was DD > ID > II. The Yo-Yo IRT 2 Test is used to determine anaerobic performance status in these sports groups (Table 3-4).

Prior research has indicated that individuals with the *ACE* DD genotype who participate in football may exhibit characteristics related to power (McAuley et al., 2021) and sprint-oriented traits (Ma et al., 2013; Ahmetov & Fedotovskaya, 2015), which aligns with the findings of our study. Several research have failed to validate these findings (Gentil et al., 2012; Ruiz et al., 2011; Garatachea et al., 2014). The results of a study investigating the athletic performance of elite and sub-elite young male football players ( $n = 73$  elite; 69 sub-elite and 107 controls), as well as the relationship between *ACE* I/D polymorphisms and performance, contradict our own findings. This study reported that *ACE* gene variants in elite and sub-elite football players do not have any association with speed, leg explosive power, and repetitive sprint ability. No significant correlation was observed between *ACE* gene polymorphisms and physical performance in research conducted on Italian and Polish athletes (Shenoy et al., 2010; Micheli et al., 2011; Massidda et al., 2012; Gentil et al., 2012; Erskine et al., 2014; Orysiak et al., 2018). In their recent study, Yıldırım et al. (2022) conducted additional research that emphasized the importance of a homogeneous sample group and the impact of lifestyle and shared environmental factors. Their findings revealed that individuals with the *ACE* gene variant (DD) initially exhibit a statistical advantage, but their performance outcomes in the long term are often similar to those with different gene variants (Yıldırım et al., 2022). These findings add to the uncertainty regarding whether *ACE* gene variants are the primary determinant of an individual's predisposition to a specific athletic activity. Genetic analysis alone is insufficient for understanding the evolution of physical performance. Other elements, such as environmental conditions, body structure, variations in skeletal muscle fiber type ratio, lifestyle, and consistent exercise, all play a significant role. The graph of athletic achievement, governed by epigenetic mechanisms, encompasses a multitude of variables. Consequently, the current data on the links between genetic differences and their interactions with certain environmental conditions are considered insufficient (Mattsson et al., 2016; Pickering et al., 2019; McAuley et al., 2021).

### Conclusion & Suggestion

This study found that non-elite male basketball and volleyball players with *ACE* ID and DD genotypes showed greater performance improvement than those with II genotypes when engaging in short-term and intermittent high-intensity maximal efforts. Additionally, the *ACE* gene polymorphisms exhibited a linear distribution, with ID  $\geq$  DD > II. In this regard, more research is needed to improve the predictability of approaches to *ACE* gene polymorphism, as the performance-enhancing effects of *ACE* gene variations on athletes can be affected by a range of genetic and environmental variables.

The drawbacks of this study include a small sample size, the inclusion of several sports branches and training conditions, and a lack of sufficient funding. For this purpose, we suggest conducting longitudinal studies with substantial sample numbers to investigate the impact of candidate genes on athletic performance.

### References

- Ahmetov, I. I., Druzhevskaya, A. M., Lyubaeva, E. V., Popov, D. V., Vinogradova, O. L., & Williams, A. G. (2011). The dependence of preferred competitive racing distance on muscle fibre type composition and ACTN3 genotype in speed skaters. *Experimental physiology*, 96(12), 1302-1310.
- Ahmetov, I. I., & Fedotovskaya, O. N. (2015). Current progress in sports genomics. *Advances in clinical chemistry*, 70, 247-314.
- Ahmetov, I. I., Vinogradova, O. L., & Williams, A. G. (2012). Gene polymorphisms and fiber-type composition of human skeletal muscle. *International journal of sport nutrition and Exercise metabolism*, 22(4), 292-303.

- Ahmetov, I. I., John, G., Semenova, E. A., & Hall, E. C. (2024). Genomic predictors of physical activity and athletic performance. *Advances in Genetics*, 1-98.
- Alvarez, R., Terrados, N., Ortolano, R., Iglesias-Cubero, G., Reguero, J. R., Batalla, A., ... & Coto, E. (2000). Genetic variation in the renin-angiotensin system and athletic performance. *European journal of applied physiology*, 82, 117-120.
- Atkins SJ. Performance of the Yo-Yo intermittent recovery test by elite professional and semiprofesinal rugby league players. *J Strength Cond Res* 2006 Feb; 20 (1): 225-5.
- Bangsbo, J, Laia, F.M and Krstrup, P. (2008). “The Yo-Yo Intermittent Recovery Test: A Useful Tool for Evaluation of Physical Performance in Intermittent Sport”. *Sports Medicine*, 38 (1), 37-51.
- Beck, K. L., Thomson, J. S., Swift, R. J., & Von Hurst, P. R. (2015). Role of nutrition in performance enhancement and postexercise recovery. *Open access journal of sports medicine*, 259-267.
- Bezuglov, E., Morgans, R., Butovskiy, M., Emanov, A., Shagiakhmetova, L., Pirmakhanov, B., ... & Lazarev, A. (2023). The relative age effect is widespread among European adult professional soccer players but does not affect their market value. *Plos one*, 18(3), e0283390.
- Carter, C. S., Onder, G., Kritchevsky, S. B., & Pahor, M. (2005). Angiotensin-converting enzyme inhibition intervention in elderly persons: effects on body composition and physical performance. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, 60(11), 1437-1446.
- Cerit, M. (2018). Evaluation of the Soldier's Physical Fitness Test Results (Strength Endurance) in Relation to Ace Genotype. *SPORT AND HEALTH International Journal of Sport Sciences and Health*, 5(9), 123-135.
- Cerit, M., Colakoglu, M., Erdogan, M., Berdeli, A., & Cam, F. S. (2006). Relationship between ace genotype and short duration aerobic performance development. *European journal of applied physiology*, 98, 461-465.
- Bulgay, C., Bayraktar, I., Kazan, H. H., Yıldırım, D. S., Zorba, E., Akman, O., ... & Mijaicá, R. (2023, February). Evaluation of the Association of VDR rs2228570 Polymorphism with Elite Track and Field Athletes' Competitive Performance. In *Healthcare* (Vol. 11, No. 5, p. 681). MDPI
- De Geus, E. J., Bartels, M., Kaprio, J., Lightfoot, J. T., & Thomis, M. (2014). Genetics of regular exercise and sedentary behaviors. *Twin Research and Human Genetics*, 17(4), 262-271.
- De Moor, M. H., Spector, T. D., Cherkas, L. F., Falchi, M., Hottenga, J. J., Boomsma, D. I., & De Geus, E. J. (2007). Genome-wide linkage scan for athlete status in 700 British female DZ twin pairs. *Twin Research and Human Genetics*, 10(6), 812-820.
- Eider, J., Cieszczyk, P., Ficek, K., Leonska-Duniec, A., Sawczuk, M., Maciejewska-Karłowska, A., & Zarebska, A. (2013). The association between D allele of the ACE gene and power performance in Polish elite athletes. *Science & Sports*, 28(6), 325-330.
- Erskine, R. M., Williams, A. G., Jones, D. A., Stewart, C. E., & Degens, H. (2014). The individual and combined influence of ACE and ACTN3 genotypes on muscle phenotypes before and after strength training. *Scandinavian journal of medicine & science in sports*, 24(4), 642-648.
- Eynon, N., Ruiz, J. R., Femia, P., Pushkarev, V. P., Cieszczyk, P., Maciejewska-Karłowska, A., ... & Lucia, A. (2012). The ACTN3 R577X polymorphism across three groups of elite male European athletes.
- Garatachea, N., Verde, Z., Santos-Lozano, A., Yvert, T., Rodriguez-Romo, G., Sarasa, F. J., ... & Lucia, A. (2014). ACTN3 R577X polymorphism and explosive leg-muscle power in elite basketball players. *International Journal of Sports Physiology and Performance*, 9(2), 226-232.
- Gao, B. (2006). Study on Relation between I/D Polymorphism of ACE Gene and Maximal Aerobic Capacity of Elite Endurance Athletes of Han Nationality in Shanghai. *SPORTS SCIENCE*, 26(2), 42.
- Gentil, P., Lima, R. M., Pereira, R. W., Mourot, J., Leite, T. K., & Bottaro, M. (2012). Lack of association of the ACE genotype with the muscle strength response to resistance training. *European Journal of Sport Science*, 12(4), 331-337.
- Goddard, N., Baker, M., Higgins, T., & Cobbold, C. (2014). The effect of angiotensin converting enzyme genotype on aerobic capacity following high intensity interval training. *International Journal of Exercise Science*, 7(3), 10.
- Goleva-Fjellet, S. (2016). ACE I/D and ACTN3 R577X polymorphisms in the Norwegian population: Do ACE I/D and ACTN3 R577X polymorphisms influence self-reported physical activity levels? (Master's thesis, Høgskolen i Telemark).
- He, C., Holme, J., & Anthony, J. (2014). SNP genotyping: the KASP assay. *Crop breeding: methods and protocols*, 75-86
- Hughes, D. C., Day, S. H., Ahmetov, I. I., & Williams, A. G. (2011). Genetics of muscle strength and power: polygenic profile similarity limits skeletal muscle performance. *Journal of sports sciences*, 29(13), 1425-1434.
- İlgün, F., Günay, V., Yıldırım, S., & Cerit, M. (2020). Atletik Performans Genleri ve Atletik Yeteneğin Belirlenmesine İlişkin Yaklaşımlar. *Turan-Sam*, 12(48).
- Jeremic, D., Macuzic, I. Z., Vulovic, M., Stevanovic, J., Radovanovic, D., Varjadic, V., & Djordjevic, D. (2019). ACE/ACTN3 genetic polymorphisms and athletic performance of female soccer players. *Revista Brasileira de Medicina do Esporte*, 25, 35-39.
- Jones, A., Montgomery, H. E., & Woods, D. R. (2002). Human performance: a role for the ACE genotype?. *Exercise and sport sciences reviews*, 30(4), 184-190.
- Kelly, A. L., & Williams, C. A. (2020). Physical characteristics and the talent identification and development processes in male youth soccer: A narrative review. *Strength & Conditioning Journal*, 42(6), 15-34.
- Kim, J. H., Jung, E. S., Kim, C. H., Youn, H., & Kim, H. R. (2014). Genetic associations of body composition, flexibility and injury risk with ACE, ACTN3 and COL5A1 polymorphisms in Korean ballerinas. *Journal of exercise nutrition & biochemistry*, 18(2), 205.
- Lightfoot, J. T., De Geus, E. J., Booth, F. W., Bray, M. S., Den Hoed, M., Kaprio, J., ... & Bouchar, C. (2018). Biological/genetic regulation of physical activity level: consensus from GenBioPAC. *Medicine and science in sports and exercise*, 50(4), 863.
- Ma, F., Yang, Y., Li, X., Zhou, F., Gao, C., Li, M., & Gao, L. (2013). The association of sport performance with ACE and ACTN3 genetic polymorphisms: a systematic review and meta-analysis. *PloS one*, 8(1), e54685.
- Massidda, M., Corrias, L., Ibba, G., Scorcu, M., Vona, G., & Calò, C. M. (2012). Genetic markers and explosive leg-muscle strength in elite Italian soccer players. *The Journal of sports medicine and physical fitness*, 52(3), 328-334.

- Mattsson, C. M., Wheeler, M. T., Waggott, D., Caleshu, C., & Ashley, E. A. (2016). Sports genetics moving forward: lessons learned from medical research. *Physiological genomics*, 48(3), 175-182.
- Mayhew, s. R., and h. A. Wenger. Time-motion analysis of professional soccer. *J. Hum. Movement Stud.* 11:49–52, 1985.
- McAuley, A. B., Hughes, D. C., Tsaprouni, L. G., Varley, I., Suraci, B., Roos, T. R., ... & Kelly, A. L. (2021). Genetic association research in football: A systematic review. *European Journal of Sport Science*, 21(5), 714-752.
- Micheli, M. L., Gulisano, M., Morucci, G., Punzi, T., Ruggiero, M., Ceroti, M., ... & Pacini, S. (2011). Angiotensin-converting enzyme/vitamin D receptor gene polymorphisms and bioelectrical impedance analysis in predicting athletic performances of Italian young soccer players. *The Journal of Strength & Conditioning Research*, 25(8), 2084-2091.
- Montgomery, H. E., Clarkson, P., Dollery, C. M., Prasad, K., Losi, M. A., Hemingway, H., ... & Humphries, S. (1997). Association of angiotensin-converting enzyme gene I/D polymorphism with change in left ventricular mass in response to physical training. *Circulation*, 96(3), 741-747.
- Montgomery, H. E., Marshall, R., Hemingway, H., Myerson, S., Clarkson, P., Dollery, C., ... & Humphries, S. E. (1998). Human gene for physical performance. *Nature*, 393(6682), 221-222.
- Moraes, V. N. D., Ferrari, G. D., Chiaratto, T., Ferezin, L. P., Trapé, Á. A., Canivarolo, A. B. P., ... & Bueno Júnior, C. R. (2016). Association of genetic polymorphisms with physical capacities and body composition in older women. *Revista Brasileira de Cineantropometria & Desempenho Humano*, 18, 11-19.
- Myerson, S., Hemingway, H., Budget, R., Martin, J., Humphries, S., Montgomery, H., & (With the Technical Assistance of Maj Mutch and Helen McGloin). (1999). Human angiotensin I-converting enzyme gene and endurance performance. *Journal of applied physiology*, 87(4), 1313-1316.
- Myerson, S. G., Montgomery, H. E., Whittingham, M., Jubbs, M., World, M. J., Humphries, S. E., & Pennell, D. J. (2001). Left ventricular hypertrophy with exercise and ACE gene insertion/deletion polymorphism: a randomized controlled trial with losartan. *Circulation*, 103(2), 226-230.
- Nazarov, I. B., Woods, D. R., Montgomery, H. E., Shneider, O. V., Kazakov, V. I., Tomilin, N. V., & Rogozkin, V. A. (2001). The angiotensin converting enzyme I/D polymorphism in Russian athletes. *European Journal of Human Genetics*, 9(10), 797-801.
- Orysiak, J., Busko, K., Michalski, R., Mazur-Różycka, J., Gajewski, J., Malczewska-Lenczowska, J., ... & Pokrywka, A. (2014). Relationship between ACTN3 R577X polymorphism and maximal power output in elite Polish athletes. *Medicina*, 50(5), 303-308.
- Papadimitriou, I. D., Lucia, A., Pitsiladis, Y. P., Pushkarev, V. P., Dyatlov, D. A., Orekhov, E. F., ... & Eynon, N. (2016). ACTN3 R577X and ACE I/D gene variants influence performance in elite sprinters: a multi-cohort study. *BMC genomics*, 17(1), 1-8.
- Pasha, M. Q., Khan, A. P., Kumar, R., Grover, S. K., Ram, R. B., Norboo, T., ... & Brahmachari, S. K. (2001). Angiotensin converting enzyme insertion allele in relation to high altitude adaptation. *Annals of human genetics*, 65(6), 531-536.
- Pescatello, L. S., Corso, L. M., Santos, L. P., Livingston, J., & Taylor, B. A. (2019). Angiotensin-converting enzyme and the genomics of endurance performance. In *Routledge Handbook of Sport and Exercise Systems Genetics* (pp. 216-250). Routledge.
- Pickering, C., Kiely, J., Grgic, J., Lucia, A., & Del Coso, J. (2019). Can genetic testing identify talent for sport?. *Genes*, 10(12), 972.
- Rankinen, T., Wolfarth, B., Simoneau, J. A., Maier-Lenz, D., Rauramaa, R., Rivera, M. A., ... & Bouchard, C. (2000). No association between the angiotensin-converting enzyme ID polymorphism and elite endurance athlete status. *Journal of applied physiology*, 88(5), 1571-1575.
- Ruiz, J. R., Fernández del Valle, M., Verde, Z., Díez-Vega, I., Santiago, C., Yvert, T., ... & Lucia, A. (2011). ACTN3 R577X polymorphism does not influence explosive leg muscle power in elite volleyball players. *Scandinavian journal of medicine & science in sports*, 21(6), e34-e41.
- Sarmento, H., Anguera, M. T., Pereira, A., & Araújo, D. (2018). Talent identification and development in male football: A systematic review. *Sports medicine*, 48, 907-931.
- Scanavini, D., Bernardi, F., Castoldi, E., Conconi, F., & Mazzoni, G. (2002). Increased frequency of the homozygous II ACE genotype in Italian Olympic endurance athletes. *European Journal of Human Genetics*, 10(10), 576-577.
- Shenoy, S., Tandon, S., Sandhu, J., & Bhanwer, A. S. (2010). Association of angiotensin converting enzyme gene polymorphism and Indian Army triathletes performance. *Asian journal of sports medicine*, 1(3), 143.
- Tucker, R., & Collins, M. (2012). What makes champions? A review of the relative contribution of genes and training to sporting success. *British journal of sports medicine*, 46(8), 555-561.
- Ulucan, K., Sercan, C., Eken, B. F., Ülgüt, D., & Erel, Ş. (2016). Spor genetiği ve ACE gen ilişkisi. *İnönü Üniversitesi Beden Eğitimi ve Spor Bilimleri Dergisi*, 3(2), 26-34.
- Williams, A. G., & Folland, J. P. (2008). Similarity of polygenic profiles limits the potential for elite human physical performance. *The journal of physiology*, 586(1), 113-121.
- Woods, D. (2009). Angiotensin-converting enzyme, renin-angiotensin system and human performance. *Genetics and sports*, 54, 72-87.
- Woods, D. R., & Montgomery, H. E. (2001). Angiotensin-converting enzyme and genetics at high altitude. *High altitude medicine & biology*, 2(2), 201-210.
- Valdivieso, P., Vaughan, D., Laczko, E., Brogioli, M., Waldron, S., Rittweger, J., & Flück, M. (2017). The metabolic response of skeletal muscle to endurance exercise is modified by the ACE-I/D gene polymorphism and training state. *Frontiers in physiology*, 8, 993.
- Yang, N., MacArthur, D. G., Gulbin, J. P., Hahn, A. G., Beggs, A. H., Eastal, S., & North, K. (2003). ACTN3 genotype is associated with human elite athletic performance. *The American Journal of human genetics*, 73(3), 627-631.
- Yıldırım, S., Koçak, M. S., Cerit, M. (2022). The Mysterious World of Genes: Physical Performance and Genetic Interactions: Traditional Review. *Türkiye Klinikleri J Sports Sci.* 2022;14(3):357-62. DOI: 10.5336/sportsci.2022-91973
- Zhao, B., Moomchala, S. M., Tham, S. Y., Lu, J., Chia, M., Byrne, C., ... & Lee, L. K. (2003). Relationship between angiotensin-converting enzyme ID polymorphism and VO<sub>2</sub>max of Chinese males. *Life sciences*, 73(20), 2625-2630.
- Zilberman-Schapira, G., Chen, J., & Gerstein, M. (2012). On sports and genes. *Recent Patents on DNA & Gene Sequences* (

