



## Association Between the ACE I/D Gene Polymorphism and Comparison of 3000 m Running Performance With and Without Equipment During the Basic Military Training

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### ABSTRACT

**Purpose:** The aim of this study is to reveal the relationship between the ACE I/D gene polymorphism in response to 3000 m running performance (both equipped and equipment-free), and the compatibility of considering the ACE gene variables as a criterion in the selection of operational duties.

**Methods:** The study group consisted of 103 non-elite Turkish army recruits. All subjects were measured for 3000 m running performance both equipped and equipment-free. Homogenous working conditions were established by providing the same type of feeding, resting and loading parameters for the subjects in the boot camp. ACE gene polymorphisms were examined by PCR method.

**Results:** According to the results of our study, while the best performance between fully equipment running performance and equipment-free running performance was observed in the subject with DD, ID and II genotype respectively. We observed that individuals with ACE DD genotype seems to be more advantageous than the ID and II genotypes in response to the fully equipped and equipment-free 3000 m running performance ( $P < 0.05$ ).

**Conclusion:** The findings in this study may be utilized for further assessments in evaluating the operational planning with regard to personnel allocation to specific tasks requiring certain level of metabolic and physical characteristics.

### Introduction

Throughout the military history, load carrying has been regarded as an important aspect of military operations. It has been the focus of many researches over the years. Technological developments of weapons and armor are designed to increase the effectiveness of the soldiers and to ensure their survival at the operational area. During training on the operational field, soldiers must be carrying loads as they travel in difficult conditions (short-medium and long distance running or marching, climbing, crawling etc.) However, the increase in load carriage due to technological developments may have negative effects on the agility and stamina of the soldiers at the battlefield.

Today, carrying heavy loads into the battlefield remains a key part of military operations. On the other hand, the increase in the load of the soldier leads to decrease in performance at the theatre [1]. Within this



framework, the increasing weight of the soldiers on their backs in trainings and operations also forces the physical exertion levels.

From a military perspective physical performance directly affects the individuals' capacity at the battlefield. Soldiers who are physically fit and higher endurance capacity can survive more easily in the battlefield. However, not only do individual goals vary, but also individuals respond and adapt to exercise differently [2]. There is increasing evidence for strong genetic influences on physical performance and for an evolutionary "trade-off" between performance traits for speed & power and aerobic/anaerobic endurance activities [3].

In recent years there has been a great progress in molecular biology techniques, which has enhanced the influence of the research both on genetics and on human performance [4]. There are specific regions of DNA that can vary among individuals. Such variations may explain why some individuals give differentiated responses to certain stimuli, including the responses to physical performance or training. In a particular sport or physical activity, the presence of specific polymorphisms may contribute to higher levels of performance [5] at the battlefield or the training area.

Basic military training is a quite challenging period of physical training for soldiers. In this framework, the development of the aerobic/anaerobic and strength endurance is the main objective of the period of basic military training for the soldiers. On the other hand, running 3000 meter with fully equipped (back bag, weapons, flasks etc) is an important theme of the the basic military training during combat phase.

In this study, in order to investigate whether the physical performance of the soldiers in fully equipped and equipment-free 3000 m running is depending on a genetic variable or not; we specifically aimed to analyze the effect of the angiotensin I-converting enzyme (ACE) gene, whose association with the human physical performance has also been extensively studied.

The ACE gene is the most frequently studied gene on the response to exercise when investigating the physical performance of the human body. It is thought to have influence on traits such as aerobic/anaerobic and strength endurance, speed, power & trainability. The ACE gene is known to have effects on the muscular force & the degree of fatigue (aerobic endurance) and adaptation to workout sessions [6]. I-allele of the ACE gene has been generally associated with improved endurance performance, while D-allele of the ACE gene has been associated with sprint and power related phenotypes [4].

ACE gene includes a polymorphism (DD, ID, and II alleles) enclosing of the availability (insertion, I) or nonentity (deletion, D) of a 287 base pair sequence in intron 16 [7,8]. comprising 26 exons and encode for ACE, which perform operative role on various substrates. ACE gene polymorphism appears to have significant mission on ACE at a tissue level [9,6]. and may effect angiotensin II production [10]. Higher ACE levels increase angiotensin II productivity (DD genotypes) and thus the increased level of plasma angiotensin II restricts the blood flow to the tissues [4].

The level of physical performance must be at a certain level for soldiers to achieve their operational duties. Running or walking for miles under difficult conditions, performing long periods of time with heavy loads, reaching targets on time, crawling for hours in challenging terrain and being able to act quickly and safely under stress in operational duties are highly complex activities requiring high physical effort. In this regard, the military field performance is dominated by aerobic endurance (low intensity, long duration activities), anaerobic endurance (high intensity, short duration activities) and strength endurance (long duration power performance without fatigue) [11].

Taking into consideration the above mentioned assessment, the aim of this study is to reveal the relationship of the ACE I/D gene polymorphism in response to 3000 m running performance (both with and



without equipment), and the ACE gene variables' being considered as a criterion in the selection of operational duties and to provide some positive contributions to the planning efforts of soldiers' training loads while considering their specific individual requirements.

## Methods

### Participants

The study group consisted of 103 male subject non-elite Turkish army recruits. All subjects have provided written consent to participate in the study and appropriate ethics committee approval has also been granted. The male subjects participating in the research study did not regularly deal with any sports activities as professionally, and homogenous working conditions were established by providing the same type of feeding, resting and loading parameters for the subjects in the boot camp. Mean age of the subjects was  $24 \pm 3.6$ , body weight was  $73 \pm 2.1$  kg, and height was  $174 \pm 2.4$  cm.

At the beginning of the boot camp, all subjects participated in 3000m running test with sportswear. And then, one week later, they run the same distance with fully equipped (a rifle G3 4250 gr, magazine 5 x 75 = 3760gr, bayonet 550 gr, battle dress uniform 1146 gr, cartridge belt 250 gr, boot 1800gr, flask 1400gr, assault vest 1140 gr, helmet 1560 gr; total weight is 15.856 kg) [12]. In general, activities have been carried out in an open and sunny weather (the average air temperature during boot camp was between 18 & 24 degrees).

### Genetic Analysis

"The genotype analysis of the subjects participated in the current study had been evaluated during the study conducted in 2004" [13], and the method used in this evaluation is explicated below.

Each subject provided with a written consent to participate in the study and appropriate ethics committee approval. Peripheral venous blood of subjects receiving the approvals were collected on K2EDTA scrapers and stored at  $-20^{\circ}\text{C}$  until used for DNA isolation. All molecular analyses were carried out in the Molecular Medicine Research Laboratory of the Department of Pediatrics, Ege University Medical Faculty Hospital.

"Genomic DNA was extracted from 200  $\mu\text{l}$  of EDTA- anticoagulated peripheral blood leucocytes using the QIAmp Blood Kit (QIAGEN, Ontario, Canada, Cat. no:51,106). Amplification of DNA for genotyping the ACE I/D polymorphism was carried out by polymerase chain reaction (PCR) in a final volume of 15  $\mu\text{l}$  containing 200 M dNTP mix, 1.5 mM  $\text{MgCl}_2$ , 1 $\times$  Buffer, 1 unit of AmpliTaq<sup>®</sup> polymerase (PE Applied Biosystems) and 10 pmol of each primer. The primers was used to encompass the polymorphic region of the ACE were 5-CTGGAGACCACTCCCATCCTTTCT-3 and 5 -ATGTGG CCATCACATTTCGTCAGAT-3"[14]. "DNA was amplified for 35 cycles, each cycle comprising denaturation at  $94^{\circ}\text{C}$  for 30 s, annealing at  $50^{\circ}\text{C}$  for 30 s, extension at  $72^{\circ}\text{C}$  for 1 min with final extension time of 7 min. The initial denaturizing stage was carried out at  $95^{\circ}\text{C}$  for 5 min. The PCR products were separated on 2.5% agarose gel and identified by ethidium-bromide staining. Each DD genotype was confirmed through a second PCR with primers specific for the insertion sequence" [15]. "The samples with II and DD homozygote genotypes and ID heterozygote genotype were randomly selected. These samples were then purified by PCR products purified system (Genomics, Montage PCR, Millipore) and directly sequenced by the ABI 310 Genetic Analyzer (ABI Prisma PE Applied Biosystems)" [16].

### Statistical Analysis

Statistical analyses were performed using SPSS for Windows version 10 (SPSS Inc., Chicago, IL, USA). Methods applied were frequencies, descriptive statistics, and means. Statistical significance was set at the  $P < 0.05$  level. The mean differences between groups those were split on two factors that are ACE genotype group and type of 3000 running (with and without full accoutered) were compared with two-way ANOVA. The post



hoc analysis was conducted with Least Significant Difference method (LSD). Lastly, the differences between running score values for participants with equipment and without equipment were observed and analyzed by paired t-test.

The effect of genotype on the difference between 3000m running time with equipment and without equipment is analyzed by using two way ANOVA. In this method, before constructing a model, the necessary assumptions whose normality and equality of variances are checked using the appropriate tests. Shapiro Wilk test satisfies the normality of samples and Levene Test shows that the equality of variances between samples ( $p=0.859$ ).

The following table shows related summary statistics for case and the result of ANOVA. The dispersion of genotypes in the entire group (16.48 % II,  $n = 16$ ; 50.47 % ID,  $n = 56$ ; 33.05 % DD,  $n = 31$ ) did not diverge from substantially from those estimated by the Hardy–Weinberg equilibrium.

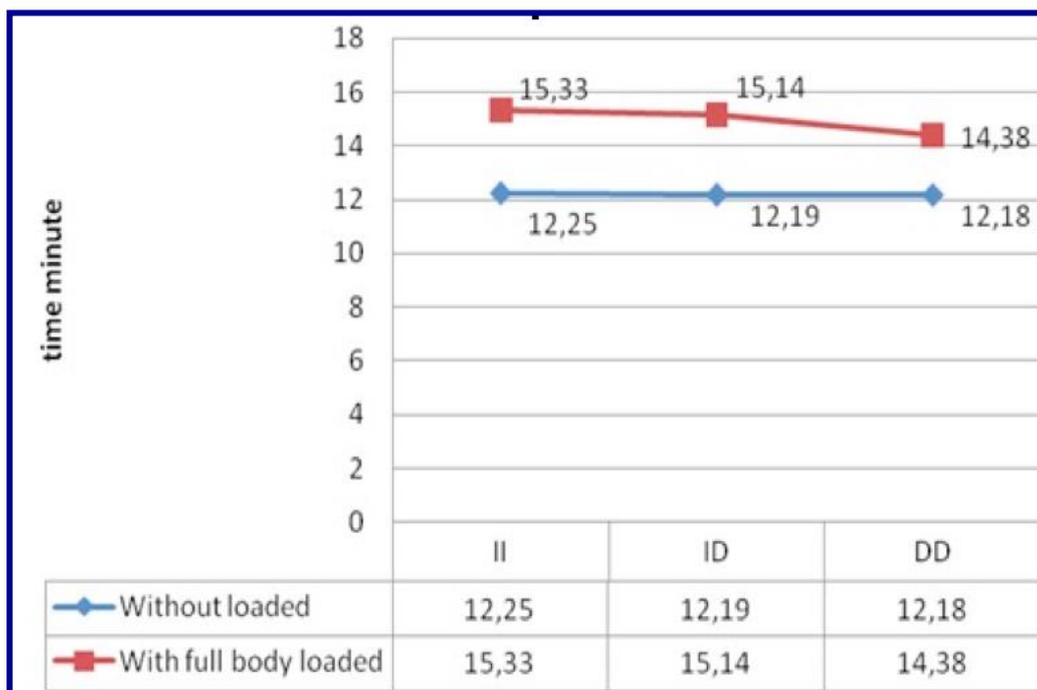
Genotype	n	Without load (n=103)	With load (n=103)	Effect of load (P value)	Genotype effect (P value)
II	16	12,25±1.28	15.33±1.25	0.000	0.005
ID	56	12,19±1.34	15,14±1.21		
DD	31	12,18±1.12	14,38±1.21		
Total	103	12,20±1.29	15,15±1.23		

**Table 1.** Two Way ANOVA (Significance level  $*P < 0.05$ ).

## Results

The mean of 3000m running time is achieved for participants with full body loaded and unloaded. It is seen that the average running scores without equipment is highest for DD, ID and II genotypes respectively, and the **table 1** shows that this difference is statistically significant ( $p=0.000$ ). In other words, it can be said that the performance of unloaded subject is better than with full body loaded on the average.

In addition, when this averages are compared in terms of genotype, it is seen that the mean running performance is best for subjects with genotype II, intermediate for subjects with genotype ID, and lowest for subjects with genotype DD.



**Table 2.** Running scores and genotype.

It is revealed that these differences among genotypes are significant ( $p=0.005$ ). In order to find out which differences are significant, Least Significant Difference (LSD) test is conducted and it is revealed that the differences between genotype DD & ID ( $p=0.006$ ) and genotype DD and II are statistically significant ( $p=0.004$ ). On the other hand, the difference for genotype ID and II is not statistically significant ( $p=0.469$ ).

Moreover, the effect of full body loaded on genotypes is examined using paired t-test. For every genotype, it is revealed that there is a significant differences between running score with & without full body loaded (**Table 2**). Participants for each genotype showed a worse performance with full body loaded. However, it can be said that the DD genotypes showed better performance than II and ID genotypes.

### Discussion and Conclusion

In literature, there is a scarcity of genetics studies related to this area, in particular the influence of ACE I/D gene on the soldiers' performance during military running or walking both with and without equipment. Majority of the works conducted have focused on the endurance capacity development, which also led to the unavailability of sufficient number of studies required to conduct a comparative study. In this sense our study is a strong candidate to be the first example of its kind. The studies conducted so far, have excluded the gene factor and mainly considered the efforts exerted by the soldiers during medium and long ranges under different loads (the loads that are carried by soldiers may differ from country to country). Besides their being focused on medium and long-range efforts, there is no available study, which also dealt and related with short-term high-intensity efforts or fast-paced (as loaded) movements or difficult terrain walking's. In this study, we aimed to study which ACE genotypes will show a better performance in reaching the end point during a 3000 m equipped military running. Taking into consideration the time as a critical factor impacting the level of success especially in the conduct of military operations or during any location changes in operations, we assumed that the ACE D genotype would present a better performance in short-term operations in comparison to other types.

There are few studies investigating the physiological reasons, and the effects of heavy load carriage during the performance of short duration, high intensity performance [17,18, 19]. During military applications in the field, a soldier will always be carrying or wearing an external load. The primary intent of physical standards in the military has always been to select soldiers best suited to the physical demands of military service [1]. The ability of the soldiers to maneuver fastly with heavy backpacks is critical for completing the mission and surviving operations. This situation becomes more difficult for the tasks performed in different environmental conditions [19].

The training programs made for the purpose of improving the mobility of the soldiers on the terrain can be listed as aerobic/anaerobic, strength and endurance, upper and lower body speed and agility trainings. All these parameters aimed to be developed through specific trainings are essential for moving in theatre with ease and comfort. However, each individual is prone to show different levels of performance depending on the varieties of their genetic specifications [4]. In other words, the aerobic or anaerobic training ability varies between individuals. It appears to be genetically determined among individuals, partly due to the composition of the muscle fiber types [20]. Normally, the fast twitch muscle fibers are important for short-duration and high-intensity work bouts, where as the slow twitch muscle fibers are better suited for sub-maximal and prolonged activities [20]. The percentage of each of these major types in a given muscle appears to be genetically determined [6].

The effects of ACE gene variables on the physical performance will be better understood in the framework of studies focusing on the relation between the ACE gene and physical performance. In our study, the metabolic and physiological characteristics of individuals with ACE DD genotype, which granted them more advantageous results in the field in comparison to ones with other types of ACE genotype, have obviously resulted from the genes that those individuals do carry. Because, ACE DD genotype is related with higher ACE activity [7], angiotensin II secretion rate & the high rate of the fast twitch muscle fiber [10, 21]. This genotype plays a key role in the development of speed & power parameter, which is the determinant of anaerobic activities [22, 23]. Cerit et al. (2006) stated that ACE DD genotype seems to have an advantage in development in short duration aerobic performance development that requires high-level  $VO_2max$ . There was also a linear trend in performance enhancement as ACE DD > ID > II [7].

Moreover, individuals with DD allele carriers showed positive improvement in  $VO_2max$  following high intensity interval training than those with II allele carrier. Furthermore, It is also stated in some studies that ACE DD genotype improves the aerobic capacity and increases the  $VO_2max$  levels and shows better performance in short duration aerobic endurance training [24, 25].  $VO_2max$  (aerobic power) levels can be sustained 10–12 min [26]. ACE DD genotypes have more performance improvement in maximal efforts, in which  $VO_2max$  is dominant and lasting between 8-10 minutes [22]. In this respect, high performance in short duration aerobic performance requires higher  $VO_2max$  and strength endurance levels [13].

On the other hand, in a number of some studies have shown that there is no relationship between ACE genotypes and  $VO_2max$  development. [27]. Sonna et al. have reported that ACE genotype was not strongly related to physical performance in their studies on the effect of training on aerobic power and muscular endurance in 147 healthy US Army recruits. [27].

Taking into consideration the above-mentioned analysis, ACE DD genotype is more effective in terms of specific bio-motor characteristics such as aerobic power, muscular strength, muscular endurance, and anaerobic power which are required to show high performance in the theatre especially during short-duration but high-intensity operations (shorter than 15 mins) [4] have a positive impact in sustained combat performance [17].

Likewise, running economy is also a significant factor in determining running performance [28]. The



weight of the body becomes important for running efficiency [26]. When determining the amount of weight the soldiers carry on the back, the person should not be more than one-third of body weight [29]. In fact, individuals with ACE II genotype, have better walking and running performance in long-term efforts (more than 30 min). However, it is a prerequisite to possess a lower and upper body strength in order to be able to proceed under high loads. Therefore, beside the aerobic/anaerobic and strength endurance, the upper and lower body strength is also an important factor in short-term high-intensity efforts conducted under heavy loads [4].

New technological development increases the physical capacity of soldiers. However, together with the technological development, the amount of load they carry on the back of the soldiers is increasing. In this regard, additional loads negatively affect the running economy of the soldiers. The studies of Cureton et al. (1978) reported that loading additional weights to the subjects (adding 5%, 10% and 15% of their body weight) decreased running performance (12 min). This distance decreased by average 89 meters on for every 5% load increase [30]. In our study, subjects with each genotype showed a poorer performance during a fully equipped 3000m running. However, DD genotypes showed better performance than II and ID genotypes (Table 1 and 2).

A survey of endurance athletes, they were dressed in weight jackets between 9% and 10 % of their body weight to investigate how they were influenced as metabolic. Lactic acid levels were significantly lower in subjects who were running with vest during submaximal running and running with additional load was found to increase the anaerobic mechanism in leg muscles [31]. Therefore, the leg muscles should have a higher strength and endurance capacity. In our study, due to the genotypes DD having higher anaerobic capacity and better performance in short duration aerobic endurance than the ID and II genotypes, their score in 3000 m running performance with and without additional load were observed as better than the others.

Running with additional weights is a condition that will affect the physical performance. Upper body and lower body strength & power are strongly related to the performance of high intensity (as in DD genotypes) military tasks with and without heavy load carriage. Daniels found that the 100g increases in shoes enhancement aerobic consumption and the performance decreased from 5: 39.17 to 5:40 minutes per mile [32]. Also, lean body weight (DD genotype has more lean body weight in comparison to ID and II genotype) is very important in determining performance in with additional loaded run [33]. However, the ACE genotypes, who have lesser muscle mass will be able to continue to run for a longer period of time. Such as lighter soldiers are more advantageous in medium and long distance aerobic endurance performance, because of lacking in extra load.

In conclusion; in our study, the average running scores without equipment is highest for DD, ID and II genotypes respectively. In other words, it can be concluded that the performance of unloaded soldiers is better than the ones with full body loaded on the average. Also, the results of our study support the study of Cam et al. (2007) [34]. The subjects with DD genotypes were more successful than the II and ID genotypes in 3000 m equipped-running which requires more strength.

Throughout the study, we observed that individuals with ACE DD genotype seems to be more advantageous than the ID and II genotypes in response to the fully equipped and equipment-free 3000 m running performance. The findings in this study may be utilized for further assessments in evaluating the operational planning with regard to personnel allocation to specific tasks requiring certain level of metabolic and physical characteristics. However, we also acknowledge that there is still much to be investigated regarding ACE genotypes and their role in not only the physical performance of individuals but also the combat relevant tasks and load carriage performances. In this respect, further research should be encouraged in order to better understand and determine the relationship between ACE gene polymorphism and its effects on load carriage of soldiers on the battlefield and evaluation of task oriented skills.

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