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THE ACE I/D GENE POLYMORPHISM AND PHYSICAL PERFORMANCE IN A NON-ELITE FEMALE COHORT

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SUMMARY

I/D polymorphism of the *ACE* gene may be associated with better endurance performance and a stronger response to exercise training. The aim of this study was to investigate the association between *ACE* gene polymorphism and athletic performance in a homogeneous female cohort. Thirty-two female non-elite Caucasian Turkish athletes with similar training backgrounds for at least six months were studied for *ACE* gene polymorphisms using PCR analysis. Their sixty-meter sprint and middle distance running test performances were evaluated. The distribution of the *ACE* I/D genotypes was found to be 34.4%, 37.5% and 28.1% for II, ID and DD polymorphisms respectively, in the study cohort. There were no significant differences amongst *ACE* genotype groups with respect to their sprint (χ^2: 3.261; p=0.196) or middle distance (χ^2: 0.396; p=0.820) performances. No significant relationship was found between *ACE* genotypes and sprint or endurance performances in non-elite female athletes. Studies with larger cohorts may produce significant relationships.

Key words: Genetics, endurance performance, insertion/deletion, sprint, exercise

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ÖZET

ELİT OLMAYAN KADIN SPORCULARDA ACE I/D GEN POLİMORFİZMİ VE FİZİKSEL PERFORMANS

ACE geni I/D polimorfizmi dayanıklılık performansı ve antrenmana daha iyi yanıtla ilişkili olabilir. Bu çalışmanın amacı ACE gen polimorfizmi ile sportif performans arasındaki ilişkiyi homojen bir grupta incelemekti. Beyaz ırktan, son altı aydır benzer antrenman geçmişine sahip olan 32 bayan, non-elit Türk sporcunun ACE gen polimorfizmleri PCR yöntemiyle analiz edildi. Performansları 60 m sprint ve orta mesafe koşu testleri ile değerlendirildi. Bulgular: Çalışma grubunu oluşturan deneklerin ACE I/D genotiplerinin dağılımı II, ID ve DD genotipleri için sırasıyla % 34.4, % 37.5 ve % 28.1 idi. ACE genotip grupları için sprint (χ^2: 3.261; p=0.196) veya orta mesafe (χ^2: 0.396; p=0.820) performansları açısından anlamlı bir fark saptanmadı. ACE genotiplerine göre sprint veya orta mesafe performansları açısından anlamlı bir farklılık bulunamadı. Daha geniş çalışma grupları ile yapılacak araştırmalar bu ilişkiyi farklı biçimde ortaya koyabilir.

Anahtar sözcükler: *Genetik, dayanıklılık performansı, ensersiyon/ delesyon, sprint, egzersiz*

INTRODUCTION

An important endocrine system controlling circulatory homeostasis is the renin-angiotensin system (RAS). The angiotensin converting enzyme (ACE) involved is released from the cell membrane, which converts angiotensin I to angiotensin II and inactivates bradykinins and tachykinins. However, local renin-angiotensin systems (and associated ACE expression) are now recognized to exist in diverse human tissues, including adipose tissue (6), skeletal muscle (3), heart (18), and lung (14).

A polymorphic variant of the human *ACE* gene has been identified as the presence (insertion, I allele) rather than the absence (deletion, D allele) of a 287 bp fragment being associated with lower serum (17) and tissue (2) ACE activity.

Previous studies have suggested that the *ACE* I-allele is overrepresented amongst elite endurance athletes (1,5,10,12), and in enhanced endurance response to training in non-elite subjects (8,15). Although yet to be fully explained, some of the association between the

ACE I-allele and endurance performance may be mediated through differences in fatigue-resistance (8,22) and muscle fiber-type distribution (23). Conversely, anaerobic performance seems to be associated with the *ACE* DD homozygous allele amongst elite athletes (10,12,21). However, other researchers have not found a relationship between *ACE* genotype and athletic performance in elite athletes (16,20), and sedentary subjects (15).

We chose to study a population of Turkish non-elite female athletes with similar training backgrounds to see whether an association exists between *ACE* gene polymorphism and physical performance.

MATERIALS AND METHODS

Subjects

Thirty-two female Caucasian non-elite Turkish athletes who were prospective students of a Sports High School, and who were trained at least for six months by their coaches and instructors to qualify for entry to the School constituted the cohort. Training documents were kindly provided by the coaches. Weekly training programs of the subjects were similar in terms of intensity, amount and type of exercise (Table 1). The study received appropriate ethics committee approval. Written informed consent was obtained from all participants.

Table 1. Training volume of subjects (n: 32) during the six-month exercise phase

Training parameters	Mean	SD
Lactate threshold (min/wk)	47.0	3.9
VO_{2max} (min/wk)	26.7	0.9
Resistance tr. (times/wk)	8.2	0.6
Sprint (m/wk)	428.2	7.5
Resistive sprint (m/wk)[1]	169.9	13.6
Plyometrics (jumps/wk)	106.4	6.1

[1]Running against resistance using elastic tubes, uphill, upstairs, etc. were included

Exercise tests

Performances for standing 60 m sprint and 1000 m middle distance runs were evaluated. A combination of two movie cameras (Panasonic NV3500EN, Japan), a movie mixer (Panasonic Production Mixer WJMX50A, Japan), and a photocell system with a timer accuracy of 0.001 sec (designed and produced at the Celal Bayar University,

School of Physical Education and Sports) were used to measure race times. Exercise tests rendered in visual images and the digital images of the timer system were superimposed. The combination of these images was recorded in order to re-evaluate actual performances.

Genetic analysis

Genomic DNA was extracted from 200 µl of EDTA-anticoagulated peripheral blood leukocyte suspension using the QIAmp Blood Kit (QIAGEN, Ontario, Canada, Cat. no: 51106). Amplification of DNA for genotyping the *ACE* I/D polymorphism was carried out by the polymerase chain reaction (PCR) in a final volume of 15 µl containing 200 µM dNTP mix, 1.5 mM $MgCl_2$, 1x buffer, 1 unit of AmpliTaq® polymerase (PE Applied Biosystems) and 10 pmol of each primer. The primers used to encompass the polymorphic region of the *ACE* gene were:

5'-CTGGAGACCACTCCCATCCTTTCT-3' and

5'-ATGTGGCCATCACATTCGTCAGAT-3' (17).

DNA was amplified for 35 cycles, each cycle comprising denaturation at 94° C for 30 sec, annealing at 50° C for 30 sec, extension at 72° C for 1 min with final extension time of 7 min. The initial denaturizing stage was carried out at 95° 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 (19). The samples with II and DD homozygote genotypes and ID heterozygote genotype were selected at random.

Statistical analysis

Statistical analyses were performed using SPSS for Windows version 11.0 (SPSS Inc., Chicago, IL, USA). Methods applied were frequencies, cross-tabulations, descriptive statistics, and means. Statistical significance was set at the $p<0.05$ level. A χ^2 test with the data read from the Finetti statistics program was used to confirm that the observed genotype frequencies were in Hardy-Weinberg equilibrium. Differences in the distributions of *ACE* genotypes according to athletic performance (60 m – 1000m) were examined using Pearson's χ^2.

RESULTS

ACE I/D genotype distribution of the group (DD 28.1%, n=9; ID 37.5%, n=12; II 34.4%, n=11) did not deviate significantly (p=0.162) from that predicted by the Hardy-Weinberg equilibrium. Although performance levels seem to decrease from II to DD arithmetically, these differences were not statistically significant (p>0.05; Pearson's χ^2; Table 2).

Table 2. Performance levels of *ACE* genotype groups (mean ± SD)

Genotype	II	ID	DD	Pearson χ^2; p
n	11	12	9	32
Sprint performance (s)	9.04 ± 0.34	9.21 ± 0.41	9.46 ± 0.61	3.26; 0.20
Middle distance performance (s)	235.7 ± 11.4	237.0 ± 17.3	246.8 ± 33.2	0.40; 0.82

DISCUSSION

Our data did not reveal any association between the *ACE* gene polymorphism and superior sprint or middle distance running performances in female subjects. We preferred to use a middle distance that could be completed between three to five min in order to monitor short duration aerobic endurance (2 to 8 min). In this type of exercise, powerful strides are necessary for better performance, which generally results in a high degree of acidosis. In addition, intense exercise that requires maximum VO_2 performance can only be sustained for eight to twelve minutes since anaerobic metabolism end products are being accumulated. Anaerobic capacity and aerobic power (VO_{2max}) are accepted to be the most important qualities for athletes in events requiring two to eight minutes of intense exertion.

Zhang et al. (23) revealed that greater the I allele frequency, higher the percentage of type-I muscle fibers, and alternatively, greater the D allele frequency, higher the percentage of type-II muscle fibers. It is known that middle distance runners have higher rates of type-II muscle fibers (48-55%), similar to weight lifters (51-56%) (13). On the other hand, the DD genotype was associated with a greater anabolic effect on cardiac (7,11) and skeletal muscles (9,11). Since exercises used in these studies were predominantly aerobic in nature, these data might have resulted from enhancement in capillary density, increased myoglobin and mitochondria content of muscle cells and elevated mitochondrial enzymes. Other studies have found the D allele to be

associated with a higher VO_{2max} (15) and greater strength gain in the quadriceps muscle in response to training (4). Furthermore, anaerobic performance also seems to be associated with DD homozygous *ACE* alleles. For example, elite short-distance (<400 meters) swimmers have greater than normal frequency of D alleles (21). These distances are completed in less than 4 minutes by elite swimmers, and anaerobic capacity and muscular endurance are very important in these events.

In many researches, excess I allele frequencies of *ACE* genes have been found to be related with higher endurance performance in elite (1,5,10,12), and non-elite subjects (8,15), or with endurance-related parameters like running economy (22) and muscular endurance (8). Many others have not pointed out any relationship between *ACE* genotype and athletic performance in either elite athletes (16,20), or sedentary subjects (15).

To conclude, no significant relationship was found between *ACE* genotypes and sprint or endurance performance in non-elite female athletes. Larger studies are required to explore the underlying mechanism of the association between I/D polymorphism and athletic status in different race and gender populations.

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