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Molecular Analysis of Genetic Variation in Angiotensin I-Converting Enzyme Gene in Turkish Athletes

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ABSTRACT Genetic factors play an important role in physical performance. The angiotensin converting enzyme (ACE) gene polymorphism is the most studied genetic marker in the field of human performance. The aim of this study was to determine the impact of ACE (I/D) polymorphism on athletic ability in Turkish athletes. Genomic DNA obtained from 237 persons (120 athletes and 117 sedentary controls) was used in the study. ACE gene I/D polymorphism genotypes were determined by using polymerase chain reaction with the specific primers. Results revealed that the ACE genotype distribution was significantly different between athletes (II 16.7%, ID 43.3% and DD 40%) and sedentary groups (II 7.7%, ID 38.5% and DD 53.8 %; P=0.036). The D allele frequency was obserwed as 38.3 % and I allele was as 61.7 % in the athlets group, it was 26.92 % and 73.08 % respectively in the control group. As a result of our study, angiotensin converting enzyme gene I/D polymorphism D allele or DD genotype could affect athletic ability in Turkish study population.

INTRODUCTION

The area of genetics has received increasing interest in sports medicine. Gene polymorphisms may contribute to explaining the inter individual variability with respect to training responses and consequently different abilities for a specific physical performance. The advanced researchs on genetics of physical performance began in the late 1990s, when the datas from Human Genom Project acquired currency (Collins 2009; Gineviciene et al. 2011). Several functional polymorphisms have been demonstrated to affect sporting phenotypes. *ACTN3* (coding for human *a*- actinin-3 gene), which has been associated with elite sprinter athletic status. There is emerging evidence that Peroxisome Proliferator Activated Receptor-Alpha (*PPAR-*α) together with Peroxisome Proliferator Activated Receptor Gamma Coactivator 1 Alpha (*PPARGC1A*) play an important role in muscle fibre type conversion (Yang et al. 2003; Moran et al. 2006; Gineviciene et al. 2011).

One of the most studied genetic markers in the field of human performance is an insertion/deletion (I/D) Alu element of the gene encoding ACE on chromosome 17. As a component of the circulating renin–angiotensin system (RAS), ACE influences circulatory homeostasis through the degradation of vasodilator bradykinin and generation of the vasopressor, angiotensin II (Ang II). Presence of the D allele has been associated with higher concentrations of circulating and tissue ACE. Increased ACE activity might lead to elevated Angiotensin II concentrations (Rigat et al. 1990). A local RAS exists in skeletal muscle and it may influence functional performance and the I allele has been associated with fatigue resistance in skeletal muscle (Montgomery and Woods 1999) and with endurance performance. In general there is an association between ACE genotype and physical performance and results suggest that allele I carriers would have advantages in cardio respiratory endurance (Calò and Vona 2008).

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In previous studies *ACE* associated with fat free mass, muscle mass and strength, and power performance. It is reported that 66% of the variance in athlete status is explained by additive genetic factors, with the remaining variance being attributable to non-shared environmental factors (De Moor et al. 2007). Montgomery and colleagues identified for the first time a positive association between a genetic variation, the insertion/deletion (I/D) polymorphism of the angiotensin-converting enzyme gene (*ACE*), and endurance exercise performance (Montgomery and Woods 1999; Eynon et al. 2009). They found that the I allele and the II genotype were overrepresented in experienced British high-altitude mountaineers (with a history of ascending beyond 7000 meters without using supplementary oxygen) compared to their healthy, non-athletic referents (Eynon et al. 2009). This polymorphism (rs. 5186) is the absence (deletion; D allele), rather than the presence (insertion, I allele) of 287bp Alu repeat element in intron 16. However, the association of *ACE* I/D polymorphism in sports abilities have been contradicted and debated (Sweta et al. 2012).

The aim of this study was to analyze the genotype distribution and allelic frequency of *ACE* gene in Turkish athletes.

MATERIAL AND METHODS

Participants

Genomic DNA obtained from 237 persons (120 athlets and 117 sedantary controls) was used in the study. The mean age ± standard deviation (SD) was 21.69 ±3.48 in athlets and 23.38 ±3.646 in control group respectively. There were 27 (22.5 %) females and 93 (77.5 %) male in athlets group and in the control group there were 26 (22.3%) females and 91 (77.7 %) male respectively. All the participiants gave informed consent and the study was approved by the ethical committee of Gaziosmanpasa University.

Genotype Determination

DNA was extracted from 2 mL venous blood according to kit procedure (Sigma, USA) and stored at -20 °C. *ACE* genotypes were determined by polymerase chain reaction (PCR). Reactions were performed with 10 pmol of each primer: sense oligo: 5'CTG GAGACCACT CCCATC CTT TCT 3'and antisense oligo: 5'GAT GTG GCC ATC ACATTC GTC AGAT 3' in a final volume of 50 ìl, containing 3 mM $MgCl_2$, 50 mM KCl, 10 mMTris-HCl pH 8.4, 0.1 mg/ml gelatin, 0.5 mM of each dNTP (Geneun, Deutschland), 2.5 U Taq DNA polymerase (Fermentas, Germany). DNA was amplified after initial denaturation (94°C, 4 min) for 30 cycles with denaturation at 94°C for 1 min, annealing at 60°C for 1 min 45 sec, and extension at 72°C for 1 min 30 sec using a thermal cycler (Techne, USA). PCR products were analyzed on 2% agarose gels after staining with ethidium bromide. In the absence of the 287 bp insertion in intron 16 of the *ACE* gene, this PCR method resulted in a 190 bp product (D allele) and in the presence of insertion, produced a 490 bp product (I allele). In heterozygous samples, 2 bands (490 and 190 bp) were detected along with a third fragment of intermediate size. In order to validate the accuracy and reproducibility of the method, PCR reaction included internal control. Second PCR was performed to confirm samples which results are not clear. Also, to confirm the accuracy of the genotyping, repeated analysis was performed on randomly selected samples. No discrepancies were found.

Statistical Analysis

Analysis of the data was performed using the computer software SPSS 15.0 (SPSS, Chicago, IL, USA) and OpenEpi Info software package program (www.openepi.com). Continuous data were given as mean ± SD (standard deviation) and (min-max). The frequencies of the alleles and genotypes in patients and controls were compared with χ^2 analysis. Odds ratio (OR) and 95% confidence intervals (CIs) were calculated. *P* value smaller than 0.05 (two-tailed) was regarded as statistically significant. Power analysis was made by using Minitab 15.0 package program. Hardy–Weinberg equilibrium was assessed by χ^2 analysis.

RESULTS

Demographic variables and baseline characteristics of athlets were given in Table 1. The mean age ± standard deviation (SD) was 21.69 ±3.48 in athlets and 23.38 ±3.646 in control group respectively. There were 27 (22.5 %) females and 93 (77.5 %) male in athletes group and in the control group there were 26 (26.2%) females and

Table 1: Demographic variables of athletes and controls.

Individual characteristics	*Athlets (n=120) (%)/(mean±SD)*	*Controls (n=117) (%)/(mean±SD)*
Sex		
Female n (%)	27 (22.50%)	26 (22.2%)
Male n (%)	93 (77.50%)	91 (77.80%)
Mean age, years [min-max]	21.69 ±3.48 [13-31]	23.38 ±3.646 [18-36]

91 (77.8 %) male respectively. Table 2 presents characteristics of athletes. Table 3 shows the distribution of *ACE* gene I/D genotypes in athlets and control groups. There was statistically significant difference between the groups with respect to *ACE* genotype distribution (p=0.036). The D allele frequency was obserwed as 38.3 % and I allele was as 61.7 % in the athlets group, it was 26.92 % and 73.08 % respectively in the control group. According to the power analysis the power of the study was over 80%. Frequency of ACE gene polymorphisms in the control group were in Hardy–Weinberg equilibrium.

Table 2: Characteristics of athletes.

Characteristics	*Athlets (n=120) (%)*
Sport Duration (years)	
<5	12 (10 %)
>5	66 (55 %)
>10	42 (35 %)
Smoking Status	
No smoking	99 (82.5 %)
<5	7 (5.8 %)
>5	14 (11.7 %)
Alchol Consumption	
No consumption	103 (85.8 %)
<5	7 (5.8%)
>5	10 (8.4 %)

DISCUSSION

Genes are responsible for about 50% of the variability in physical performance and in the response to training (Calò and Vona 2008). Genetic studies in the field of sport science is very recent and is constantly increasing. Molecular genetic tests based on DNA technologies are actively used in sports genetics to assess the human predisposition to different physical features. For example, some genes have been found to be associated with speed and power characteristics and endurance performance (Voroshin and Astratenkova 2008). The angiotensin converting enzyme (*ACE*) polymorphism is the most studied genetic marker in the field of human performance (Voroshin and Astratenkova 2008).

In the present study, there was a statistically significant difference between the groups with respect to *ACE* genotype distribution (p=0.036). The D allele frequency was obserwed as 59.05 % and I allele was as 40.95 % in the athletes group, it was 41.75 % and 58.25 % respectively in the control group. In a previous study Williams et al. (Williams et al. 2002) examined the ACE I/D genotype associations with quadriceps muscle strength in 81 young Caucasian men. Baseline isometric strength was significantly associated with the ACE genotype, with I-allele homozygous showing the lowest strength values (Gentil et al. 2012). Ginevience et al. provided evidence that the *ACE* DD genotype was significantly lower and *ACE* ID genotype higher for all football players compared with the controls (Gineviènienë et al. 2011). Massida et al.'s

Table 3: The distribution of *ACE* gene I/D genotypes and alleles is in athletes and control groups.

Genotype	*Athlets (n=120) (%)*	*Controls (n=117) (%)*	χ^2	*P value*	*OR (95%CI)*
DD	48 (40)	63 (53.8)	6.668	0.036	
II	20 (16.7)	9 (7.7)			
ID	52 (43.3)	45 (38.5)			
ID+II: DD	72:48	54:63	4.561	0.032	1.75 (1.045-2.93)
DD+ID:II	100:20	108:9	4.443	0.035	0.4167 (0.181-0.957)
Allele frequency					
D	92 (38.3)	63 (26.92)	7.009	0.008	1.687 (1.144-2.489)
I	148 (61.7)	171 (73.08)			

The results that are statistically significant are typed in bold.

results suggest a lack of association between the *ACE* I/D polymorphism and elite gymnastics performance in Italians. Some association studies (Gayagay et al. 1998; Nazarov et al. 2001) have suggested that the I allele was more common among endurance athletes because it confers a metabolic advantage during such events (Massidda et al. 2011). Scot et al. conclude that *ACE* I/D and A22982G polymorphisms are not strongly associated with elite endurance athlete status amongst Kenyans (Voroshin and Astratenkova 2008; Scott et al. 2005).

The *ACE* DD genotype may be related to better short-duration aerobic endurance performance (Kothari et al. 2012). They reported that the ACE I/D polymorphism may not be considered a marker for human performance in Indian population. Kothari et al. revealed no association between *ACE* genotype and sporting abilities (Kothari et al. 2012). A similar finding has been observed in the study carried out by Oh et al. (2007) in Korean male elite athletes, as well as Kenyan endurance athletes (Scott et al. 2005). However, association of the II genotype has been demonstrated in Italian Olympic endurance athletes. In contrast to this study, Amir et al., reported the deletion allele 'D' to be associated with the likelihood of being an endurance athlete in their Israeli cohort (Amir et al. 2007). Though there have been multiple studies on association of *ACE* gene variation in endurance sports, the results have been contradictory. In one study, it was demonstrated that an excess of I allele in elite middle distance Russian athletes (event duration 1-20 min) while another study reported an association of D allele in Israeli elite endurance athletes (Nazarov et al. 2001). Gonzales et al reported that the *ACE* gene polymorphism does not influence erythropoietic activity in endurance athletes after short-term exposure to moderate altitude (González et al. 2006). Tobina et al. suggest that the D allele of the *ACE* gene I/D polymorphism is associated with a high level of human endurance (Tobina et al. 2007). Athletes with the homozygous II genotype can run a longer distance than equally well-trained athletes with other ACE genotypes (Voroshin and Astratenkova 2008). Another interesting study related *ACE* polymorphisms with the type and the efficiency of skeletal muscle fibers (Zhang et al. 2003; Guadalupe et al. 2011). There is an association between the *ACE* II genotype and an increased percentage of type I skeletal muscle fibers (slow-twitch fibers), compared with the DD genotype (Calò and Vona 2008).

CONCLUSION

The present study suggest that, angiotensin converting enzyme gene I/D polymorphism D allele or DD genotype could affect athletic ability in Turkish study population. Larger sample sizes for functional studies are necessary to further substantiate these findings.

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