Medicina Sportiva
Med Sport 14 (2): 73-76, 2010
DOI: 10.2478/v10036-010-0014-5

NO ASSOCIATION BETWEEN ACE I/D VARIATION AND ENDURANCE PERFORMANCE LEVEL IN MEXICAN MARATHON RUNNERS

Juan R. López-Taylor[1(B,E,G)], Juan L. Martínez[2(B,G)], Celia M. Quiñónez[4(E,F)], Thomas D. Fahey[3(E)], Miguel A. Rivera[4(A,C,D,E)]

[1]Physical Activity and Applied Sport Sciences Institute, University of Guadalajara, Jalisco, México
[2]Sport Medicine and Applied Sciences Unit, Sinaloense Sports Institute, Sinaloa, México
[3]Dept of Kinesiology, California State University, Chico, CA, USA
[4]Department of Physical Medicine, Rehabilitation and Sports Medicine, School of Medicine, University of Puerto Rico, San Juan, Puerto Rico

Abstract

Introduction and aim of the study: We examined the association between the ACE insertion (I) / deletion (D); (I/D) genotype and endurance performance level in Mexican marathon runners.

Materials and methods: DNA samples were obtained from 437 subjects (men n = 231; women n = 206) who had completed at least two 42 Km road races. Cases; CAS (n = 199; men = 111; women = 88) were finishers in the top 3rd percentile, while controls; CON (n= 238; men = 142; women = 96) were those in the lowest 3rd percentile of their respective age and gender. The DNA sequence variation was detected by the tetra-primer ARMS-PCR procedure. Genotypes were determined by PCR product size.

Results: The three expected ACE genotypes were observed in both groups (CON: DD = 27%, ID = 49%, II = 24%; and CAS: DD = 28 %, ID = 41%, II = 31%). These genotype frequencies were in Hardy-Weinberg equilibrium (X^2, P>0.05) and were not significantly different between genders or groups (X^2, P>0.05). Allelic frequencies (CON; D = 0.51%, I = 0.49% and CAS; D = 0.49%, I = 0.51) were not significantly different between groups or genders (X^2, P>0.05). Comparing genotype frequencies by I or D allele carrier status revealed no statistically significant differences (X^2, P>0.05).

Conclusions: We found no support for the hypothesis of an association between the ACE insertion/deletion polymorphism and endurance performance level in a homogeneous cohort of Mexican marathon runners

Key words: *genetics, chromosome 17, athletes, marathon running, endurance, case-control*

Introduction

The association between the Angiotensin I Converting Enzyme (ACE) genotype and endurance performance (EP) is a matter of strong debate. The ACE gene, 17q23, displays 26 exons along 21-kilobases [1, 2]. The ACE gene's most studied DNA sequence variation is an insertion (I)/deletion (D) polymorphism in intron 16 [3]. The I allele or D allele consist on the presence or absence of a 287 base pair (bp) Alu-sequence. The highest and lowest mean levels of serum ACE seems to occur in subjects homozygote's for the D and I allele, respectively [4].

This protein is part of the renin-angiotensin system, which is a homeostatic mechanism that regulates blood pressure and extra-cellular fluid volume [5, 6]. ACE acts in the process of transforming Angiotensin I to Angiotensin II and in the inactivation of bradykinin and tachykinins [6]. Angiotensin is a vasoconstrictor while bradykinin and tachykinins are vasodilators. Angiotensin also antagonizes glucose uptake in skeletal muscle [5]. The ACE genotype-dependent variation in ACE activity may influence EP mainly by altering circulatory homeostasis and skeletal muscle metabolism [7-9]. Many studies [8, 10-14] have examined the association between the ACE I/D genotype and EP in various ethnic groups. Such efforts have led to conflicting results. Several reports [8, 10, 11, 13, 14] showed an association between ACE I/D polymorphism and EP phenotypes, while other studies did not [15-19]. To date, the association between the ACE gene 287 bp I/D polymorphism at intron-16 and EP level has not been investigated in a large cohort of healthy Hispanics endurance athletes. The purpose of this study was to examine the allelic and genotypic frequency distribution of the ACE gene 287 bp I/D polymorphism at intron-16 and to explore its association with EP level in healthy Mexican marathon runners.

Materials and methods

Subjects

This investigation was a case-control study. The subjects were ethnically matched, biologically unrelated

Table 1. *Characteristics of 437 biologically unrelated Mexican marathon runners*

Group	Gender	Age (years)	Height (cm)	Weight (kg)
Cases (N=199)	Men (n=111)	31 ± 9	178 ± 10	60 ± 13
	Women (n=88)	29 ± 14	161 ± 12	52 ± 12
Controls (N=238)	Men (n=142)	37 ± 13	174 ± 7	64 ± 11
	Women (n=96)	30 ± 11	158 ± 11	50 ± 10

Values are mean ± standard deviation

volunteers, who were recruited during marathon competitions conducted between 2004 and 2008 in Mexico. The competitions included the Gran Maratón Pacifico, Mazatlán, México; the Guadalajara International Marathon, Guadalajara, México; and the Marathon of Culiacán, Culiacán, México. In all instances, the study received the approval by the Medical Commissions from each of the competitions. Written informed consent was obtained from all subjects. The physical characteristics of the cases and controls are presented in Table 1.

Since the ACE genotype distribution is influenced by ethnicity [20], the recruitment process included an interview to determine ethnic and geographic area of origin and place of residence. The interview also determined history of participation in endurance events, medical history and use of medications. Any subject with a history of cardiovascular, respiratory, arthritis, metabolic disease or who took medications to control these diseases was excluded from the study. Subjects were also excluded if they reported any incident that negatively affected their performance during the race (e.g., significant injury). The subjects' classification as cases or controls was based on the official results of the competitions.

Cases (n = 199; men = 111; women = 88) were adult Mexican marathon runners who finished in the top 3rd percentile of their respective age and gender group in at least one of the competitions. Controls (n = 238; men = 142; women = 96) were adult Mexican marathon runners who finished in the lowest 3rd percentile of their respective age and gender group.

DNA Isolation

Genomic DNA was purified from whole blood by means of the Promega Wizard Genomic DNA Purification Kit (Promega Corporation, Madison, WI). The detailed procedures were described previously [21].

Genotype determination

The ACEI/D polymorphism was genotyped according to a procedure described previously [3]. The detection of the 287 bp I/D polymorphism was performed by the tetra-primer Amplification Refractory Mutation System Polymerase Chain Reaction (ARMS-PCR) procedure [22] using primers that span the I/D site and gave PCR products of 490 bp for the insertion allele and 190 bp for the deletion allele. Further procedures were described elsewhere [21]. All the work (DNA extraction, storage and management, as well as PCR and genotyping) was performed at the Exercise Physiology and Genetics Laboratory of the Department of Physical Medicine, Rehabilitation and Sports Medicine at the School of Medicine of the University of Puerto Rico, San Juan.

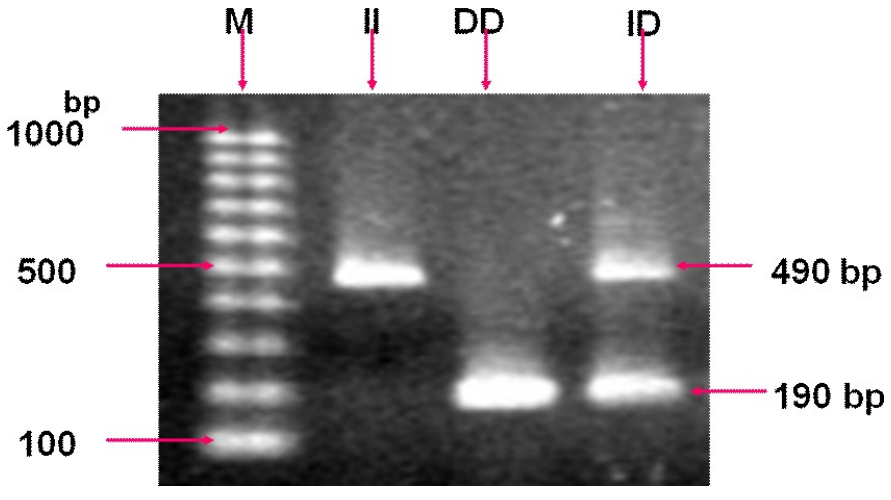

Figure 1. *Migration of ACE insertion (I) and deletion (D) fragments in 1.5% agarose gel. bp = base pair; M = 100 bp ladder marker; II = homozygote for Insertion allele; DD = homozygote for Deletion allele; ID = heterozygote.*

Statistical analysis

Statistical analyses were done using the Epi Info 3.5.1 software package [23]. *P* values ≤0.05 were statistically significant. A Chi-square test was used to confirm that the observed genotype frequencies were in a Hardy-Weinberg equilibrium (HWE) and to compare the *ACE ID* allele and genotype frequencies between athletes and controls, as well as between genders. HWE describes a state in which genotype frequencies are constant from generation to generation and in which genotype frequencies are a product of allele frequencies. This condition prevails in situations in which there are an absence of mutations, migrations, non-random mating and environmental factors favoring particular genotypes.

Results

Genotype frequencies. The three expected ACE I/D genotypes were observed in both groups and genders. In the cases and controls, these genotype frequencies, within each gender, were in **HWE** ($\chi 2$, $P \geq 0.05$) and were not significantly ($\chi 2$, $P \geq 0.05$) different between genders (not shown). Since there were no differences in the observed genotypic frequency distributions within genders, for both cases and controls, the data for both genders were pooled. The pooled data in Table 2 showed no significant difference in the genotype prevalence distribution between the cases and controls ($X^2 = 3.37$, df=2. P=0.19). The genotype frequencies by I or D allele carrier status were not significantly different (X^2, P>0.05; Table 3).

Allelic frequencies. The observed allelic frequency distributions (Table 2) within the cases as well as controls revealed no gender differences (X^2, $P \geq 0.05$). Chi square test on the gender pooled allelic frequency distribution (cases; D = 0.49%, I = 0.51; and controls; D = 0.51%, I = 0.49%) revealed no significant differences ($X^2 = 0.64$, df = 1, $P = 0.425$) between cases and controls.

Discussion

We tested whether the *ACE ID* genotype was associated with EP levels in a homogeneous sample of 437 Mexican marathon runners. Our findings showed no association between ACE I/D genotype as well as for the I or D allele carrier status. As in the present study, others [17] reported no gender differences in ACE I/D allelic frequencies. Likewise, some reports [15, 16] indicated no differences between cases and controls for the ACE I/D allelic frequencies. In those studies, no associations were found between the ACE I/D polymorphism and endurance athlete status or cardiorespiratory endurance performance. However, Myerson and co-workers [8] reported an association between the incidence of I allele with elite endurance performance, while Amir et al. [11] reported an elevated frequency of the D allele in Israeli elite marathon runners.

In our homogeneous cohort of Mexican marathon runners, we found no association between the observed ACE genotypes (i.e., DD, ID, II) and marathon performance. The present findings are in agreement with many that found no association between the ACE genotype and EP [15-19]. This study challenges the findings of others that suggested that the ACE II genotype favors EP [7-9] and those that found a

Table 2. *Absolute and relative (%) allele and genotype frequencies for the ACE I/D polymorphism for 437 biologically unrelated Mexican marathon runners*

	Genotypes*			Alleles**	
Group	DD (%)	DI (%)	II (%)	D (%)	I (%)
Cases*** (n=199)	56 (0.28)	82 (0.41)	61 (0.31)	194 (0.49)	204 (0.51)
Controls**** (n=238)	64 (0.27)	117 (0.49)	57 (0.24)	245 (0.51)	231 (0.49)

*$X^2 = 3.37$, df=2. P=0.19; **$X^2 = 0.64$ df=1, P=0.42;
***Hardy-Weinberg equilibrium (X^2, $P \geq 0.08$);
****Hardy-Weinberg equilibrium (X^2, $P \geq 0.70$)

Table 3. *Prevalence of ACE genotype frequency by D and I allele carrier status in biologically unrelated Mexican marathon runners*

	Cases	Controls
D Allele carriers	0.69	0.76
I Allele carriers	0.72	0.73

higher frequency of the ACE DD genotype in athletes of endurance requiring sports [11, 12].

In conclusion, the present report did not support an association between the ACE I/D polymorphism and endurance performance level in a homogeneous sample of Mexican marathon runners. Since ACE genotype distribution is influenced by ethnicity [20], it is not likely that our results were influenced by variations in ethnic origin.

Acknowledgements

Funding received from CRC-RCMI 1P20RR11126 and RCMI #G12RR03051.

Refrerences:

1. Mattei M-G, Hubert C, Alhenc-Gelas F, et al. Angiotensin-i converting enzyme gene is on chromosome 17. *Cytogenet Cell Genet* 1989; 51: 1041.
2. Soubrier F, Alhenc-Gelas FO, Hubert C, et al. Two putative active centers in human angiotensin i-converting enzyme revealed by molecular cloning. *Proc Natl Acad Sci USA* 1988; 85: 9386-90.
3. Rigat B, Hubert C, Alhenc-Gelas F, et al. An insertion/deletion polymorphism in the angiotensin i-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest* 1990; 86: 1343-6.
4. Lindpaintner K, Pfeffer MA, Kreutz R, et al. A prospective evaluation of an angiotensin-converting-enzyme gene polymorphism and the risk of ischemic heart disease. *N Engl J Med* 1995; 332: 706-11.
5. Dietze GJ, Henriksen EJ. Angiotensin-converting enzyme in skeletal muscle: Sentinel of blood pressure control and glucose homeostasis. *J Renin Angiotensin Aldosterone Syst* 2008; 9: 75-88.
6. Wang P, Fedoruk MN, Rupert JL. Keeping pace with ace: Are ace inhibitors and angiotensin ii type 1 receptor antagonists potential doping agents? *Sports Med* 2008; 38: 1065-79.
7. Lucia A, Gomez-Gallego F, Chicharro JL, et al. Is there an association between ace and ckmm polymorphisms and cycling performance status during 3-week races? . *Int J Sports Med* 2005; 26: 442-7.
8. Myerson S, Hemingway H, Budget R, et al. Human angiotensin i-converting enzyme gene and endurance performance. *J Appl Physiol* 1999; 87: 1313-6.
9. Tsianos G, Sanders J, Dhamrait S, et al. The ace gene insertion/deletion polymorphism and elite endurance swimming *Eur J Appl Physiol* 2004; 92: 360-2.
10. Alvarez R, Terrados N, Ortolano R, et al. Genetic variation in the renin-angiotensin system and athletic performance. *Eur J Appl Physiol* 2000; 82: 117-20.
11. Amir O, Amir R, Yamin C, et al. The ace deletion allele is associated with israeli elite endurance athletes. *Exp Physiol* 2007; 92: 881-6.
12. Ginevičienë V, Kučinskas V, Kasnauskienë J. The angiotensin-converting enzyme gene insertion/deletion polymorphism in lithuanian professional athletes. *Acta Medica Lituanica* 2009; 16: 9-14.
13. Montgomery H, Clarkson P, Barnard M, et al. Angiotensin-converting-enzyme gene insertion/deletion polymorphism and response to physical training. *Lancet* 1999; 541-5.
14. Montgomery H, Marshall R, Hemingway H, et al. Human gene for physical performance. *Nature* 1998; 393: 221-2.
15. Rankinen T, Wolfarth B, Simoneau J-A, et al. No association between the angiotensin-converting enzyme id polymorphism and elite endurance athlete status. *J Appl Physiol* 2000; 88: 1571-5.
16. Scott RA, Moran C, Wilson RH, et al. No association between angiotensin converting enzyme (ace) gene variation and endurance athlete status in kenyans. *Comp Biochem Physiol A Mol Integr Physiol* 2005; 141: 169-75.
17. Sonna LA, Sharp MA, Knapik JJ, et al. Angiotensin-converting enzyme genotype and physical performance during us army basic training. *J Appl Physiol* 2001; 91: 1355-63.
18. Taylor RR, Mamotte CDS, Fallon K, et al. Elite athletes and the gene for angiotensin-converting enzyme. *J Appl Physiol* 1999; 87: 1035-7.
19. Rankinen T, Perusse L, Gagnon J, et al. Angiotensin-converting enzyme id polymorphism and fitness phenotype in the heritage family study. *J Appl Physiol* 2000; 88: 1029-35.
20. Barley J, Blakwood A, Carter ND, et al. Angiotensin converting enzyme insertion/deletion polymorphism: Asssociation with ethnic origin. *J Hypertens* 1994; 12: 955-7.
21. Martínez JL, Carrión A, Florián ME, et al. Aquaporin-1 gene DNA variation predicts performance in hispanic marathon runners. *Med Sport* 2009; 13: 251-5.
22. Ye S, Dhillon S, Ke X, et al. An efficient procedure for genotyping single nucleotide polymorphisms. *Nucl Acids Res* 2001; 1-8.
23. Harbage B, Dean A. Distribution of epi info software: An evaluation using the internet. *Am J Prev Med* 1999; 16: 314-7.

Received: March 13, 2010
Accepted: April 20, 2010
Published: April 27, 2010

Address for correspondence:
Miguel A. Rivera
Department of Physical Medicine, Rehabilitation & Sports Medicine
Main Building Office A204
School of Medicine, University of Puerto Rico
San Juan, P.R. 00936
787-751-9625 (Phone)
787-754-1471 (Fax)
email: miguel.rivera10@upr.edu; miguelrivera@prtc.net

Juan R. López-Taylor: taylor@cucs.udg.mx
Juan L. Martínez: jl_mtz12@hotmail.com
Celia M. Quiñónez: celiamqg@hotmail.com
Thomas D. Fahey: discusdoc@aol.com