Medicina Sportiva
Med Sport 13 (4): 219-223, 2009
DOI: 10.2478/v10036-009-0034-1

LACK OF AN ASSOCIATION BETWEEN CKMM GENOTYPE AND ENDURANCE PERFORMANCE LEVEL IN HISPANIC MARATHON RUNNERS

Juan L. Martínez[1(B,E,G)], Shayan Khorsandi[2(E,F)], Robert Sojo[2(B,F)], Cristina Martínez[2(B,F)], José A. Martín[3(B,G)], Juan R. López-Taylor[4(B,G)], Thomas D. Fahey[5(E)], Miguel A. Rivera[2(A,C,D,E)]

[1] Sección de Medicina y Ciencias Aplicadas al Deporte, Instituto Sinaloense del Deporte, Sinaloa, México
[2] Department of Physical Medicine, Rehabilitation and Sports Medicine, School of Medicine, University of Puerto Rico, San Juan, Puerto Rico
[3] Dept of Physiotherapy, Faculty of Medicine, Universidad San Pablo CEU, Madrid, Spain
[4] Instituto de Ciencias Aplicadas a la Actividad Física y al Deporte,Universidad de Guadalajara, Jalisco, México
[5] Dept of Kinesiology, California State University, Chico, CA, USA

Abstract

Introduction and aim of the study: Genetics plays a key role in determining endurance performance. We examined the association between a genetic polymorphism at the muscle-specific creatine kinase (CKMM) locus and endurance performance level (EPL) in elite Hispanic marathon runners.

Materials and methods: DNA samples were successfully genotyped from 380 Hispanic marathon runners (men n = 220; women n=160). Cases (n=190; men=110, women=80) were finishers in the top 3 percentile, while controls (n=190; men=110, women=80) were those in the lowest 3 percentile of their respective age and gender. Genotypes were determined by PCR and digestion with NcoI endonuclease.

Results: The three expected genotypes for the CKMM-NcoI were observed in the cases and controls. These genotypes frequencies, within each gender, were in Hardy-Weinberg equilibrium (X^2, $P \geq 0.05$) and were not significantly (X^2, $P > 0.05$) different between genders. There was no gender difference ($P \geq 0.05$) in the allelic frequencies. The pooled data for both genders revealed no significant differences in genotype ($P \geq 0.05$) or allele ($P \geq 0.05$) frequencies between cases and controls.

Conclusions: These results replicate the findings of a previous study in Caucasians (Med Sci Sports Exerc. 1997; 29:1444-47) and suggest that the CKMM-NcoI polymorphism is not an important determinant of endurance performance level.

Key words: *genetics, chromosome 19, athletes, aerobic capacity, running*

Introduction

The present study is part of a larger investigation aimed at the identification of genes contributing to *elite* endurance performance in Hispanics. Several studies support an association of the muscle-specific creatine kinase (CKMM) gene with endurance performance related phenotypes (1-5). The CKMM gene has been mapped to the q13.2-q13.3 region of chromosome 19 (6). This gene extends over 17.5 kilobase pairs and contains 8 exons and 7 introns (7).

Recent reports indicate that 262 single nucleotide polymorphisms (SNP's) are known in this gene (8). Of these SNPs an A/G DNA variation, in the 3'untranslated region (9), formerly identified as a NcoI restriction site (5, 10, 11) has shown an association with aerobic power in the sedentary state and its response to endurance training (4, 5) as well as with running economy responses to an 18-week 5000-m training program (2).

Creatine kinase (CK) is an abundant enzyme in skeletal muscle and the heart (12, 7). The cytosolic muscle isoform of the enzyme is one of at least four subunits of CK that are known for their tissue-specific expression (7, 13, 14). Some of the isoenzymes of creatine kinase are muscular (CK-MM), brain (CK-BB) and two mitochondrial (CK-MB) subunits (ubiquitous and a sarcomeric) (12). Each is associated to particular regions of high-energy demand within the cell's cytoplasm.

The CKMM isozyme generates high concentrations of ATP in the region of the myosin heads (13). It is bound specifically to the M-line of the myofibril (15) sub fragment one of heavy meromyosin in the vicinity of the myosin ATPase (16), and to the sarcoplasmic reticulum outer membrane and vesicles (17). CKMM plays a key role in energy homeostasis of cells with intermittently high, fluctuating energy requirements. The enzyme CK catalyzes a near equilibrium, dead-end reaction that ensures that the ATP/ADP ratio is maintained. The reaction catalyzed is $MgATP + Cr \Leftrightarrow PCr + MgADP + H$. Although this is the general reaction for most of the CK, CKMM catalyzes a re-

versible transfer of the N-phosphoryl group from the phosphocreatine (PCr) to ADP to regenerate ATP (12). Such reactions take place whenever ATP production by oxidative phosphorylation or glycolysis does not match the ATP utilization rates.

The fact that CKMM is expressed in cardiac and skeletal muscle and it is associated with endurance performance-related phenotypes suggest a possible relationship with endurance performance. The aims of the present work were to determine the allelic frequencies and genotype prevalence for a single nucleotide polymorphism (A → G) in the 3' untranslated region of the CKMM gene (9) and to investigate the association of this genetic polymorphism with endurance performance level in a broad Hispanic cohort.

Methods

This investigation was designed as a case-control study. The subjects were biologically unrelated volunteers who competed in at least one of the following long distance running events between 2004 and 2008: Marathon of Madrid, Madrid, Spain; Gran Maratón Pacifico, Mazatlán, México; Guadalajara International Marathon, Guadalajara, México; Marathon of Culiacán, Culiacán, México. Gender specific values for the physical characteristics for the cases and controls are presented in Table 1.

The recruitment process included an interview to determine the following matching and/or inclusion/exclusion criteria: ethnic and geographic area of origin, place of residence, history of participation in endurance events, cardiovascular disease, respiratory disease, arthritis activity, history of metabolic disease, and prescribed medications. Any subject with known history of cardiovascular, respiratory, arthritis, metabolic disease or on medication was excluded from this report. Subjects who reported incidents that negatively affected his/her race outcome were also excluded from the study. Subject classification as either case or control was based on the official final results of the event.

Cases

Subjects (n = 190; men= 110; women=80) were adult Hispanic marathon runners who finished in the top 3rd percentile of their respective age and gender group in at least one of the above long distance running events. Controls (n = 190; men=110; women=80) were adult Hispanic marathon runners who finished in the lowest 3rd percentile of their respective age and gender. The Medical Commissions from each of the endurance events approved the study, and written informed consent was obtained from all subjects.

DNA Isolation

Genomic DNA was purified from whole blood by means of the Promega Wizard Genomic DNA Purification Kit (Promega Corporation, Madison, WI). Briefly, 20 milliliters of venous peripheral blood was collected from each subject, in tubes containing EDTA. The first step in the purification procedure required the lysis of the red blood cells as well as the white blood cells and their nuclei. An RNase digestion step followed. The cellular proteins were removed by a salt-precipitation step leaving the high molecular weight genomic DNA in solution. Next, the genomic DNA was concentrated and desalted by isopropanol precipitation. Finally, the extracted DNA was rehydrated in 800 µl of Promega DNA Rehydration Solution (10mM Tris-HCl (pH 7.4) and 1mM EDTA (pH 8.0)) and stored at -80°C (Forma Scientific Freezer 916, Marietta, Ohio). The concentration and quality, as evaluated by the absorbance ratio at 280 vs. 260 nm, of the DNA was determined by spectrophotometry.

Genotype determination

The studied polymorphism (restriction site number = 1803285 (8), is an A/G DNA variation in the 3'untranslated region (9), formerly identified as a NcoI restriction site (5, 10, 11). The polymerase chain reaction (PCR) (18) was performed in a GeneAmp PCR system 9800 (Applied Biosystems GeneAmp 9800, Foster City, CA). Primers were as follows: 5' GTG-CGG-TGG-ACA-CAG-CTG-CCG 3' and 5' CAG-CTT-GGT-CAA-AGA-CAT-TGA-GG 3' (5, 10). Total volume of the PCR was 25-µL of a reaction mixture containing Qiagen Taq master mix (Qiagen Inc, Ventura, CA), 0.3 µM of each forward and backward primers, and 5 – 10 ng of genomic DNA. The amplification protocol was: 1) one cycle of denaturation at 95°C for 5 min; 2) 30 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s, and extension at

Table 1. *Characteristics of 380 biologically unrelated marathon runners of the Hispanic Gene Study*

Group	Gender	Age (years)	Height (cm)	Weight (kg)
Cases (N=190)	Men (n=110)	33 ± 10	177 ± 8	61 ± 6
	Women (n=80)	28 ± 12	160 ± 9	50 ± 10
Controls (N=190)	Men (n=110)	38 ± 12	172 ± 5	64 ± 7
	Women (n=80)	31 ± 14	159 ± 10	48 ± 12

Values are mean ± standard deviation

72°C for 45 s; and 3) one final 5 min elongation cycle at 72°C. Preventive contamination measures were taken by the inclusion of PCR reaction mixture without DNA (negative control) in every run of amplification. After each amplification, the PCR product was digested with 5 U of *NcoI*. Restriction digest conditions were those recommended by the enzyme manufacturer (New England Biolabs, Ipswich, MA). The resulting fragments were separated by horizontal electrophoresis on 1.5% agarose gels. Each gel was run for 60 min at 150 mA while refrigerated at 10°C, stained with ethidium bromide, and photographed under UV transmitted lights *using a Bio-Rad Gel-Doc 2000 system.* A 100 bp ladder was used as a length marker to estimate the size of the digested DNA fragments. The allele without the *NcoI* restriction site was designated as allele A (1170 bp), whereas the allele with the polymorphic *NcoI* site was designated as allele G (985 and 185 bp). According

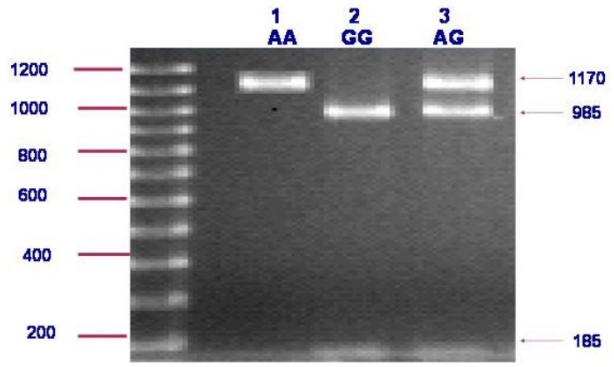

Figure 1. *Migration of CKMM Nco I fragments in 1.5% agarose gel*

to the digestion pattern, subjects were scored as GG when homozygous (985 + 185 bp) for the presence of the restriction site, AA when homozygous (1170 bp) for the absence of the restriction site, and AG in the case of heterozygosity (1170, 985 and 185bp).

After all the genotyping work was completed, a systematic random sample of DNA aliquots was performed. The selected DNA samples were submitted to a new round of PCR and genotyping as described above. In case of a mismatch between the first and second genotyping, the sample was resubmitted to a PCR and genotyping cycle. DNA extraction, storage, and management, as well as PCR and genotyping were 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.

Statistical analysis

The Pearson Chi-square test was used to examine differences in allele frequencies between genders, cases vs. controls and to determine whether the genotype prevalence distribution differed from that expected under the Hardy-Weinberg equilibrium. The polymorphism information content (PIC) and the heterozygosity index (H) were calculated according to procedures described elsewhere (19, 20) The PIC (20) value is an indicator of the probability that a marker locus is informative. It is estimated from the frequency of heterozygotes that are determined by the number of marker alleles and their respective frequencies. H (19) is a probability measure of the likelihood that a randomly selected subject is heterozygous for any two alleles at a given gene locus. Odds ratios with 95% confidence interval as well as the Chi-square tests were performed using the Epi Info 3.5.1 software package (21). *P* values <0.05 were considered statistically significant.

Results

Allele and genotype frequencies

The three expected genotypes for the CKMM-NcoI polymorphism at the 3' untranslated region were observed in the Cases and Controls (Table 2). In both groups of Cases and Controls, these genotype frequencies, within each gender, were in Hardy-Weinberg equilibrium (X^2, $P \geq 0.05$) and were not significantly (X^2, $P \geq 0.05$) different between genders. The observed allelic frequencies were within the range of those pre-

Table 2. *Absolute and relative (%) allele and genotype frequencies for the NcoI polymorphism in the 3' untranslated region of the muscle-specific creatine kinase gene for 380 biologically unrelated marathon runners of the Hispanic Gene Study*

	Genotypes*			Alleles**		Odds Ratio	95% Confidence Interval	*P* Value
Group	CC (%)	CA (%)	AA (%)	C (%)	A (%)			
Cases*** (n=190)	15 (0.08)	67 (0.35)	108 (0.57)	97 (0.26)	283 (0.74)	0.83	0.60 – 1.16	0.08
Controls**** (n=190)	20 (0.11)	71 (0.37)	99 **(0.52)**	111 (0.29)	269 (0.71)			

* $X^2 = 1.22$, df=2. P=0.54
** $X^2 = 1.30$ df=1, P=0.26
*** Hardy-Weinberg equilibrium (X^2, $P \geq 0.05$)
**** Hardy-Weinberg equilibrium (X^2, $P \geq 0.05$)

viously reported for Caucasians (22). In both Cases and Controls the 1170 bp allele was the less frequently observed. There was no gender difference ($P \geq 0.05$) in the allelic frequencies. The pooled data for both genders revealed no significant differences in genotype prevalence ($P \geq 0.05$) or allele ($P \geq 0.05$) frequencies between the Cases and Controls.

Degree of polymorphism

The observed measures of the degree of polymorphism at the CKMM-*NcoI* polymorphic site for the Cases and Controls are presented in Table 3. The PIC values and H index were within the range of other published data for non-Hispanics (22).

Table 3. *Polymorphism information content (PIC) and Heterozygozity index (H) for 380 biologically unrelated marathon runners of the Hispanic Gene Study*

Group	PIC	H
Cases (N=190)	0.38	0.31
Controls (N=190)	0.41	0.33

Discussion

The present study determined the allelic frequencies and genotype prevalence for a SNP (A/G) in the 3' untranslated region of the CKMM gene (9) and examined its association with endurance performance level in a broad Hispanic cohort. The main finding of the study revealed no statistical differences in the observed allelic and genotypic frequencies of the CKMM A/G polymorphism between cases and controls.

To our knowledge, the present study represents the first attempt to examine the association of the present CKMM A/G SNP with endurance performance level. Two previous publications (23, 22) had examined the same CKMM marker and its association with $\dot{V}O_2$max (22), as well as performance status (23). In one case-control design (22) the allelic frequencies and genotype prevalence for the CKMM SNP were examined in Caucasian males subjects with a high (79 ± 4 mL·kg^{-1}·min^{-1}) and low (30 ± 9 mL·kg^{-1}·min^{-1}) $\dot{V}O_2$max. The findings of that study indicated that the A/G *SNP* at the CKMM gene did not contribute to the proper classification of subjects into high or low $\dot{V}O_2$max status. Along those lines, in another case-control study (23), the association between the CKMM SNP and performance status was examined in professional cyclists, sedentary controls, and Olympic class runners. No statistical differences were found for the CKMM SNP prevalence between the three groups.

We should not ignore the fact that the CKMM A/G SNP has shown significant associations with the $\dot{V}O_2$max (4, 5) and running economy (2) responses to endurance training. In humans, previous studies indicate that $\dot{V}O_2$max, a common indicator of endurance exercise capacity and positively correlated with endurance performance, was associated with the CKMM SNP under study (4, 5). Furthermore, a low but statistically significant genetic linkage was observed between the present CKMM SNP and the $\dot{V}O_2$max response to endurance training (4). Those finding revealed a consistent association between the CKMM 3' untranslated region A/G SNP gene in close proximity and the response of the $\dot{V}O_2$max to endurance training in biologically unrelated healthy adults. Another recent study (2) revealed a statistically significant association between the polymorphism in the 3' untranslated region of the CKMM gene and running economy response to endurance training.

In conclusion, the findings of the present study broadened the scope of information that suggest how a gene can influence one endurance performance-related phenotype but not another. Endurance performance as well as endurance performance status/level are two highly complex phenotypes in which no major gene effect has yet been identified. It can be contended that the CKMM or close by gene plays a role in the response of the aerobic capacity to endurance training but it does not shed light regarding endurance performance status or level. Further research seems warranted to elucidate such a contention. Finally, the present findings do not provide support to the hypothesis of an association between the CKMM 3' untranslated region A/G polymorphism and endurance performance level in our cohort of Hispanics.

Acknowledgement

Supported by CRC-RCMI 1P20RR11126, RCMI #G12RR03051, Maratón Pacifico, Maratón de Culiacán, Maratón de Guadalajara, Maratón Popular de Madrid.

References

1. Brancaccio P, Limongelli FM, Maffulli N. Monitoring of serum enzymes in sport. *Br J Sports Med* 2006; 40: 96-7.
2. Zhou DQ, Hu Y, Liu G, et al. Muscle-specific creatine kinase gene polymorphism and running economy responses to an 18-week 5000-m training programme. *Br J Sports Med* 2006; 40: 988-91.
3. Echegaray M, Rivera MA. Role of creatine kinase isoenzymes on muscular and cardiorespiratory endurance: Genetic and molecular evidence. *Sports Med* 2001; 31: 919-34.
4. Rivera MA, Pérusse L, Simoneau J-A, et al. Linkage between a muscle-specific CK gene marker and $\dot{V}O_2$max in the HERITAGE family study. *Med Sci Sports Exerc* 1999; 31: 698-701.
5. Rivera MA, Dionne FT, Simoneau J-A, et al. Muscle-specific creatine kinase gene polymorphism and $\dot{V}O_2$max in the heritage family study. *Med Sci Sports Exerc* 1997; 29: 1311-7.
6. Nigro JM, Schweinfest CW, Rajkovic A, et al. cDNA cloning and mapping of the human creatine kinase M gene to 19q13. *Am. J Hum Genet* 1987; 40: 115-25.
7. Trask RV, Strauss AW, and Billadello JJ. Developmental regulation and tissue-specific expression of the human muscle creatine kinase. *J Biol Chem* 1988; 263: 17142-9.

8. National Center for Biotechnology Information, U.S. National Library of Medicine [Internet]. Bethesda (MD): Entrez Single Nucleotide Polymorphism; [cited 2006 May 25]. Available from: http://preview.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=1803285.
9. Heled Y, Bloom MS, Wu JT, et al. CK-MM and ACE genotypes and physiological prediction of the creatine kinase response to exercise. *J Appl Physiol* 2007; 10: 1-25.
10. Gennarelli M, Novelli G, Cobo A, et al. 3' creatine kinase (M-type) polymorphism linked to myotonic dystrophy in italian and spanish populations *Hum Genet* 1991; 87: 654-6.
11. Coerwinkel-Driessen M, Schepens J, van Zandvoort P, et al. Ncoi RFLP at the creatine kinase-muscle type gene locus (CKMM, chromosome 19). *Nucl Acids Res* 1988; 16: 8743.
12. Wallimann T. Some new aspects of creatine kinase (CK): Compartmentation, structure, function and regulation for cellular and mitochondrial bioenergetics and physiology. *Biofactors* 1998; 8: 229-34.
13. Wallimann T, Wyss M, Brdiczka D, et al. Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: the „phosphocreatine circuit" for cellular energy homeostasis. *Biochem J* 1992; 281: 21-40.
14. Haas RC, and Strauss AW. Separate nuclear genes encode sarcomere-specific and ubiquitous human mitochondrial creatine kinase isoenzymes. *J Biol Chem* 1990; 265: 6921-7.
15. Turner DC, Wallimann T, and Eppenberger HM. A protein that binds specifically to the m-line of skeletal muscle is identified as the muscle form of creatine kinase. *Proc Nat Acad Sci USA* 1973; 70: 702-5.
16. Botts J, Stone DB, Wang ATL, et al. Electron paramagnetic resonance and nanosecond fluorescence depolarization studieson creatine-phosphokinase interaction with myosin and its fragments. *J Supramol Struc* 1975; 3: 141-5.
17. Rossi AM, Eppenberger HM, Volpe P, et al. Muscle-type MM cretine kinase is specifically bound to sarcoplasmic reticulum and can support Ca^{2+} uptake and regulate local ATP/ADP ratios. *J Biol Chem* 1990; 265: 5258-66.
18. Mullis K, Faloona F, Scharf S, et al. Specific enzymatic amplification of DNA in vitro: The polymerase chain reaction. *Cold Spring Harb Symp Quant Biol* 1986; 51 Pt 1: 263-73.
19. Ott J. Genes and genetic polymorphisms. In: *Analysis of human genetic linkage.* Baltimore, MD: The John Hopkins University Press, 1991, p. 23-38.
20. Botstein D, White RL, Skolnick M, et al. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am J Hum Genet* 1980; 32: 314-31.
21. Dean AG, Arner TG, Sunki GG, et al. Epi info™, a database and statistics program for public health professionals. Centers for Disease Control and Prevention. Atlanta GA, USA, 2007.
22. Rivera MA, Dionne FT, Wolfarth B, et al. Muscle-specific creatine kinase gene polymorphisms in elite endurance athletes and sedentary controls. *Med Sci Sports Exerc* 1997; 29: 1444-7.
23. Lucia A, Gómez-Gallego F, Chicharro LJ, et al. Is there an association between ACE and CKMM polymoprhisms and cycling performance status during 3-week races? *Int J Sports Med* 2005; 26: 442-7.

Received: September 13, 2009
Accepted: November 20, 2009
Published: December 03, 2009

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, Puerto Rico 00936
787-751-9625 (Phone)
787-754-1471 (Fax)
Email: miguel.rivera10@upr.edu

Juan L. Martínez: jl_mtz12@hotmail.com
Shayan Khorsandi: shayan_khorsandi06@hotmail.com
Robert Sojo: airman140@yahoo.com
Cristina Martínez: cristina.martinez@upr.edu
José A. Martín: jamurria@ceu.es
Juan R. López-Taylor: taylor@cucs.udg.mx
Thomas D. Fahey: discusdoc@aol.com