THE INFLUENCE OF ACE I/D GENE POLYMORPHISM IN AMATEUR AMERICAN FOOTBALL ATHLETES IN BRAZIL

A INFLUÊNCIA DO POLIMORFISMO GENÉTICO I/D DA ECA EM ATLETAS AMADORES DE FUTEBOL AMERICANO NO BRASIL

LA INFLUENCIA DEL POLIMORFISMO GENÉTICO I/D DE LA ECA EN ATLETAS AMATEUR DE FÚTBOL AMERICANO EN BRASIL

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ABSTRACT

Introduction: Physical performance depends on a variety of biological and mechanical properties. These different phenotypes are related through the complex interaction between the environment and the individual genetic profile. The hypothesis is that there is a hereditary component that interferes in physical fitness. ACE stands out among the genes that may influence this response. Objectives: The objective of this study is to analyze the polymorphism of the ACE gene in American football athletes. Methods: At the end of the study, the sample was composed of 45 male athletes and 72 non-athletes. DNA was extracted from the jugal mucosa. ACE polymorphisms were genotyped through polymerase chain reaction and analyzed using the electrophoresis process. To compare the frequency of genotypes between athletes and the control group, we used the Chi-square test. The association between the frequencies of alleles was verified through the 2X2 contingency tables analyzed using the Chi-square test with Yates correction. The type of study was diagnostic - Investigation of a diagnostic test, level of evidence II. A p-value of ≤0.05 was considered statistically significant for all the analyses. Results: The results showed a greater frequency of the D allele in American football athletes when compared with non-athletes, and a significant difference in the genotypic distribution of the athletes being composed of a higher number of the DD genotype as compared to the control group. Conclusion: The study provides evidence of the allelic and genotypic influence of ACE polymorphism in amateur American football players in Brazil. ***Level of evidence II; Investigation of a diagnostic test.***

Keywords: Genetics; Sports training; Physical functional performance.

RESUMO

Introdução: O desempenho físico depende de uma série de propriedades biológicas e mecânicas. Esses diferentes fenótipos são relacionados através da complexa interação entre o ambiente e o perfil genético individual. A hipótese é que existe um componente hereditário que interfere na aptidão física. O gene da ECA destaca-se entre os genes que podem influenciar nessa resposta. Objetivos: O objetivo do presente estudo consiste em analisar o polimorfismo genético da ECA em atletas de futebol americano. Métodos: Ao final do estudo, a amostra foi composta por 45 atletas do sexo masculino e 72 não atletas. O DNA foi extraído da mucosa jugal. A genotipagem dos polimorfismos da ECA foi realizada pela reação em cadeia da polimerase e analisada utilizando o processo de eletroforese. Para comparar a frequência dos genótipos entre os atletas e o grupo controle foi utilizado o teste Qui-quadrado. A associação entre as frequências dos alelos foi verificada através das tabelas de contingência 2X2 analisadas usando o teste Qui-quadrado com correção de Yates. O tipo de estudo foi diagnóstico – Investigação de um teste diagnóstico, nível de evidência II. Um valor de p ≤ 0,05 foi considerado estatisticamente significativo para todas as análises. Resultados: Os resultados apresentaram uma frequência maior do alelo D nos atletas de futebol americano quando comparados com os não-atletas e uma diferença significativa na distribuição genotípica dos atletas composta por um maior número do genótipo DD *confome comparado ao grupo controle. Conclusão: O estudo apresenta a evidência da influência alélica e genotípica do polimorfismo da ECA em atletas amadores de futebol americano no Brasil.* ***Nível de evidência II; Investigação de um teste diagnóstico.***

Descritores: *Genética; Treinamento esportivo; Desempenho físico funcional.*

RESUMEN

Introducción: El desempeño físico depende de una serie de propiedades biológicas y mecánicas. Estos diferentes fenotipos son relacionados a través de la interacción compleja entre el ambiente y el perfil genético individual. La hipótesis es que hay un componente hereditario que interfiere en la aptitud física. El gen de la ECA se destaca entre los genes que pueden influir en esta respuesta. Objetivos: El objetivo del presente estudio consiste en analizar el polimorfismo genético de la ECA en atletas de fútbol americano. Métodos: Al final del estudio, la muestra estaba compuesta por 45 atletas masculinos y 72 no atletas. El ADN se extrajo de la mucosa yugal. La genotipificación de los polimorfismos de ECA se realizó mediante la reacción en cadena de la polimerasa y se analizó mediante el proceso de electroforesis. Para comparar la frecuencia de los genotipos entre los atletas y el grupo control se utilizó el test Chi-cuadrado. La asociación

entre las frecuencias de los alelos se verificó a través de las tablas de contingencia 2X2 analizadas mediante el test de Chi-cuadrado con la corrección de Yates. El tipo de estudio fue diagnóstico-Investigación de un test diagnóstico, nivel de evidencia II. Para todos los análisis, se consideró estadísticamente significativo un valor de p ≤0,05. Resultados: Los resultados presentaron una mayor frecuencia del alelo D en los atletas de fútbol americano, en comparación con los no atletas, y una diferencia significativa en la distribución genotípica de los atletas compuesta por un mayor número del genotipo DD, conforme comparado al grupo control. Conclusión: El estudio presenta la evidencia de la influencia alélica y genotípica del polimorfismo de ECA en los atletas amateur de fútbol americano en Brasil. ***Nivel de evidencia II; Investigación de un test diagnóstico.***

Descriptores: *Genética; Entrenamiento deportivo; Rendimiento físico funcional.*

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INTRODUCTION

Physical performance depends on a variety of biological and mechanical properties. Such properties may be metabolic or anatomical, such as strength, cardiorespiratory capacity, tendon length and elasticity, muscle tension properties and fiber properties. These different phenotypes are related through the complex interaction between the environment and the individual genetic profile. The hypothesis is that a hereditary component interferes with physical fitness besides being able to interact with environmental factors and with physical training particularly.[1]

Discussions about genetics and sports performance have been made since the 1950s. However, studies have received increasing attention only in the 1970s and 1980s.[2]

Until the 1990s, the study of these complex traits was based on analysis of twins, families, and association studies. Prud'homme et al. (1984) indicated in their study that there was almost eight times as much variation in VO_2max (maximal volume of oxygen) between pairs of dizygotic twins when compared to pairs of monozygotic twins. Furthermore, monozygotic twins responded similarly to training when compared to dizygotics.

In addition, it has been possible to investigate complex traits such as physical performance,[1,3,4] making the creation of the "Human Gene Map for Health Performance and Fitness Phenotypes" possible, which reported 221 autosomal genes,[3] among which one of the most acknowledged is the insertion (I) / deletion (D) polymorphism in the gene angiotensin converting enzyme (ACE I / D).

The gene encoding the angiotensin converting enzyme (ACE I / D) has a polymorphism consisting of 287 base pair absence (deletion, D) or presence (insertion, I).[4] The allele I seems to be associated with resistance events while the D allele has been shown to be related to strength / power events.[5] The D allele is associated with higher ACE activity in both plasma and tissues,[6] thus, homozygotes for the D allele (DD genotype) show higher ACE activity when compared to the genotypes ID and II.

ACE was first associated with performance by Montgomery et al. in 1998, and was the first genetic variant to be related to physical performance. Results showed that the I allele presented higher frequency in mountaineers, while the D allele presented lower frequency when compared to the control. The genotype II patients obtained better results in a post-training muscular resistance test compared to the other genotypes (ID and DD). Since then ACE polymorphism has gained attention from researchers in the field of physical activity and sport. Authors have shown significant results of D allele hegemony with force / power events,[7] as in a study in which a sample composed of elite European swimmers of various distances showed excess of the D allele when compared to the control group. The greater presence of the D allele was maintained when only the swimmers of smaller distances (400 m or less) were analyzed, but the same did not occur with the greater distances.[8] American Football is characterized by a variety of physical abilities, being the most relevant, strength and power.[9] In this scenario, it is possible that there is a favorable genetic profile for this sport. Thus, the study becomes relevant in order to contribute to the understanding of the importance of the ACE I / D polymorphism with the performance in American Football. The aim of the study was to analyze the ACE I / D gene polymorphism in amateur American football players in Brazil and to compare the genotypic and allelic frequency with the non-athlete control population.

SUBJECTS AND METHODS

Subject characteristics

The sample consisted of 45 male amateur American Football players for the athletes group and 72 non-athletes between males and females for the control group, all from the state of Paraná, who were previously informed (WICF) about the study. These subjects signed a written informed consent form in addition to a survey form about their morphological profile (weight, height, how long they practice the sport).

The inclusion criteria used in the present study were: (1) athletes in training for more than 1 year; (2) have signed the WICF and answered the survey form. On the other hand, the athletes who wished to freely abstain from performing any procedure related to data collection for the research were excluded. All procedures were approved by the research ethics committee of under the opinion of number 1,137,045.

Collection of samples and extraction of genomic DNA

The cells were collected from the scraping of the mucous jugal and the mouthwash with 3% glucose solution. Subsequently, the samples were centrifuged and the extraction buffer added (TRIS 10 mM, EDTA 4 mM, SDS 0.5% pH 7.76). The samples were then digested overnight with proteinase K (20 mg / ml). DNA was separated from cell debris by density gradient centrifugation using ammonium acetate (8M in 1mM EDTA). The genomic DNA was then precipitated with isopropyl alcohol and 70% ethyl alcohol, isolated by centrifugation and finally resuspended in elution buffer (10 mM TRIS; 1 mM EDTA, pH 7.76). DNA quantification was performed using a spectrophotometer (NanoK, Kasvi, 2015), and the concentration was adjusted to 1 mg / mL for subsequent storage at -20 °C.

Genotyping

A genotyping of the I / D polymorphism of the ACE gene was performed by the PCR (Polymerase Chain Reaction) technique. The I / D polymorphism of the ACE gene consists of absence (deletion or "D" allele) or presence ("I" insertion or allele) of 287 base pairs without intron 16. Thus, part of institute 16 was amplified primers: direct 5'-CTGGAGAGCCACTCCCATCCTTTCT-3 'and reverse 5'-GATGTGGCCATCACATTCGTCAGAT-3'.[4] For each reaction, 1x Buffer for Taq, 3.0 mM MgCl2, 0.2 mM dNTP, 1.0 μM of each primer, 1 unit of Taq DNA polymerase and 100 ng of genomic DNA as template were used, totaling 25 μL. For amplification of the genetic material and used in the following program: 95 °C for 5 minutes of initial denaturation and enzyme release, 30 cycles of

denaturation at 94 ° C for 30 seconds, annealing at 57 ° C for 1 minute, extension one 72 °C for 1 minute. After 30 cycles, 5 minutes final extension at 72 °C. The amplicons were separated by 1% agarose gel electrophoresis and developed with 5 µg / mL ethidium bromide. The D allele of the ACE gene generates an amplicon of 191 base pairs, while the I allele generates an amplicon of 478 base pairs, containing a 287 bp insert.

According to the literature, erroneous classification of D / I heterozygotes as D / D homozygotes may occur due to preferential amplification of the D allele and amplification inefficiency of the I allele.[10] Therefore, to increase specificity of genotyping, samples showing D / D genotype were re-evaluated by a new PCR using a specific primer specific for the insertion: 5'-TTTGAGACGGAGTCTCGCTC -3' [10] and the primer reverse 5'-GATGTGGCCATCACATTCGTCAGAT-3'. The results were visualized after 1% agarose gel electrophoresis and revealed with 5 µg / ml ethidium bromide. The appearance of a band of 408 base pairs is indicative of the presence of the I allele, that is, the previously genotyped D / D samples are classified as I / D.

Survey form

The questionnaire was composed of open questions, in which participants answered their name, age, height, weight and how long they play.

Statistical analysis

The Pearson's Chi-square test was used. The associations between the allele frequencies were verified through 2X2 contingency tables analyzed by the Yates correction chi-square test. All analyzes were performed in SPSS 20.0 software. The level of significance of $p \leq 0.05$ was considered.

RESULTS AND DISCUSSION

It is observed in Table 1 that there was no significant difference between the ages of the control group and the group of athletes.

In the present study, a significant difference was observed regarding the DD genotype of the athletes group (51.1%) and the control group (29.2%) $p < 0.05$. The same significance was found when we compare the allelic distribution of ACE I / D, (68.8%) and the control group (55.5%), $p < 0.05$ (Table 2 and 3). Therefore, it is assumed that there is relevance of this polymorphism with the compatibility of the physical qualities required in the modality. It is worth mentioning that such association can be better evidenced in an experiment that presents a larger sample, corroborating the relevance of the results obtained in this study.

Table 1. Sample characteristics.

Group	N	Age
Control	72	22,19±4,76
Athletes	45	25,94±7,12

Table 2. Genotypic distribution of ACE I / D.

Group	D/D	I/D	I/I
Control	21 (29,2%)	38 (52,7%)	13 (18,1%)
Athletes	23 (51,1%)	16 (35,5%)	6 (13,4%)

$p < 0,05$.

Table 3. Allelic distribution of ACE I / D.

Group	D	I
Control	80 (55,5%)	64 (44,5%)
Athletes	62 (68,8%)	28 (31,2%)

$p < 0,05$.

The results of the present study show that there is a greater representation of the D allele and the ACE DD genotype in amateur American football athletes when compared to non-athlete population. These findings are in line with other studies, which show the relationship of ACE DD genotype to strength and power sports such as American Football.[8,11]

Studies show that the greater presence of the D allele is associated with strength / power athletes, making the carriers of this allele, especially the DD genotype, superior in tests associated with these phenotypes, by increased levels of ACE and, therefore, angiotensin II, which can influence the hypertrophic response to exercise,[12] or the likely effect of ACE I / D polymorphism on the proportion of muscle fibers.[13] These effects may be explained by the association of the D allele with the increase of angiotensin II, a growth factor[14] and the reduction of bradykinin,[15] a growth inhibitor.[16]

The correlation between D allele and power sports is different from that of allele I and resistance performance, which is likely to be associated with better muscle efficacy.[7] As was shown in non-trained subjects, a relative association between ACE levels with isometric and isokinetic strength, and D / D homozygotes showed higher strength indicators than I / I homozygous.[17] On the other hand, a study with military, ACE I / D polymorphism has been associated with metabolic efficiency, subjects with genotype I / I increased metabolic action by 1.87% versus a reduction of 0.30% in D / D genotype after 11 weeks of endurance training.[18]

One of the possible reasons for this significant representation of the ACE polymorphism in American football athletes would be due to the level of these participants, who are athletes of a team that is considered to be one of the best in Brazil, in high level athletes.[19] This was evidenced by Nazarov et al. (2001), in their study in which only elite swimmers showed an excess of the D allele, implying that the same study with teams of a lower level may demonstrate different results.

CONCLUSION

This study demonstrates the influence of the ACE I / D gene is evidenced in American football athletes, showing that subjects with D / D genotype have a predisposition to be successful when participating in this sport, as expected for a sport of strength and power. Proving that athletes with I / I genotype were found on the study in a smaller proportion when compared to the control group, indicating that the difficulty of athletes with I / I genotype to became American football players, possibly because them do not respond well to stimuli of strength and power.

Today coaches take into account for the elaboration of a periodization the variables such as time, intensity, density and complexity; in contrast, recent studies found that genetics may be a pre-determining factor in responding to training loads and thus should be an aspect to be considered. This way, athletes can receive specific training based on their own genotype, making them successful at any sport they may choose.

A better understanding of sports-related genetics can help coaches create more specific and functional trainings for each of their athletes. In this way, the understanding of cellular efficiency factors has important applications beyond the world of sports.

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