ACTN-3 and ECA genes expression do not influence the acute change in muscle mechanical and functional properties in youth handballers

Randall Gutiérrez-Vargas[1,2], Jose Alexis Ugalde-Ramírez[1,2], Guillermo Miranda[1,2], Isabel Briceño-Suarez[1,2], Rocío Ulloa-Sandí[1,2], Daniel Rojas-Valverde[1,2,3]

[1]Centro de Investigación y Diagnóstico en Salud y Deporte (CIDISAD), Escuela Ciencias del Movimiento Humano y Calidad de Vida (CIEMHCAVI), Universidad Nacional, Heredia Costa Rica. [2]Núcleo de Estudios para el Alto Rendimiento y la Salud (NARS), Escuela Ciencias del Movimiento Humano y Calidad de Vida (CIEMHCAVI), Universidad Nacional, Heredia Costa Rica. [3]Clínica de Lesiones Deportivas (Rehab&Readapt), Escuela Ciencias del Movimiento Humano y Calidad de Vida (CIEMHCAVI), Universidad Nacional, Heredia Costa Rica.

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Summary

The purpose of this study was to explore the potential relationship between ACTN-3 and ACE gene expression over the change in muscle mechanical and functional properties in youth handballers through a congested tournament. 30 players of the first handball division of Costa Rica participated in this study. The participants played a national tournament during three consecutive days (one match per day). The collection of genetic samples was through a mouth rinse with a 5% sucrose solution before the tournament. PCR tests were used to detect the alleles of the ACE and ACTN3 genes and the product´s reaction was visualized by electrophoresis. Before and after each match, tensiomyography (TMG) and Countermovement jump (CMJ) tests were used to assess mechanical and functional properties respectively. Descriptive frequency analyses and a one-way analysis of variance of independent groups were the statistics test applied. The results showed that the most prevalent polymorphisms expression was ACTN-3 R-X (56.7%) and ECA I-D (43.3%). No significant differences (p> 0.050) were found between genes expressed in the mechanical responses (contraction time (TC), delay time (TD) and, maximum radial displacement (DM)) of the rectus femoral muscle of the dominant leg neither in performance in the test CMJ. Likewise, there was no significant change (p> 0.050) in muscle mechanical or functional properties post official matches. In conclusion, handball players have the genes ACE and ACTN. Nevertheless, it seems to have no influence of these genes on the mechanical or functional muscles acute responses. More investigations will be needed to explain and understand the real impact of this gene's expression on muscle performance in handball players.

Key words:
Sport. Muscles. Genes. Physical functional performance. Genetics.

Expresiones de los genes ACTN-3 y ECA no influyen en el cambio agudo de las propiedades musculares mecánicas y funcionales en jugadores juveniles de balonmano

Resumen

El propósito de este estudio fue explorar la relación potencial entre la expresión ACTN-3 y ACE sobre el cambio en las propiedades musculares mecánicas y funcionales de jugadores juveniles de balonmano a través de un torneo congestionado. Participaron 30 jugadores de la primera división de balonmano de Costa Rica. Los participantes jugaron un torneo nacional durante tres días consecutivos. La recolección de muestras genéticas se realizó mediante un enjuague bucal con una solución de sacarosa al 5% antes del inicio del torneo. Pruebas de PCR fueron usadas para detectar los aleros de los genes ACTN-3 y ACE y la reacción del producto fueron visualizadas por electroforesis. Antes y después de cada partido, se utilizaron las pruebas de tensiomiografía (TMG) y de salto contramovimiento (CMJ) para evaluar las propiedades mecánicas y funcionales respectivamente. Las pruebas estadísticas aplicadas fueron análisis descriptivo de frecuencias y un análisis de varianza de una vía para grupos independientes. Los resultados mostraron que la expresión de polimorfismos más prevalente fue ACTN-3 R-X (56,7%) y ECA I-D (43,3%). No se han encontrado diferencias significativas (p> 0,050) entre genes expresados en las respuestas mecánicas (tiempo de contracción (TC), tiempo de retardo (TD) and, máximo desplazamiento radial (DM)) del músculo recto femoral de la pierna dominante ni en el rendimiento en la prueba de CMJ. Asimismo, no hubo cambios significativos (p> 0.050) en las propiedades mecánicas o funcionales de los músculos después de los partidos. En conclusión, los jugadores de balonmano tienen los genes ACE y ACTN, sin embargo, parece que estos genes no influyen en las respuestas agudas mecánicas o funcionales de los músculos. Se necesitan más investigaciones para explicar y comprender el impacto real de la expresión de estos genes en el rendimiento muscular de los jugadores de balonmano.

Palabras clave:
Deporte. Músculo. Genes. Rendimiento físico funcional. Genética.

Correspondencia: Randall Gutiérrez-Vargas
E-mail: randall.gutierrez.vargas@una.ac.cr

Introduction

The study of genetic factors within sports science has been relevant due to their influence on sports performance[1,2]. Genetics has long been used to understand individual performance capacities in different sports[3]. Genetic polymorphisms are associated with muscular strength and aerobic capacity, improving sports performance in training and competition[4,5]. Within performance in elite sports, a more complex phenotypic composition is needed, which is why it is influenced by anatomical, biochemical, and psychological factors[2,6]. Likewise, genetic profiles offer a piece of valuable information that can help talent identification[7].

The high-level sport requires strength directly related to muscle fibres; these respond specifically from enzymatic activity, muscle contraction, morphology and metabolism[1]. To these responses, the angiotensin-converting enzyme (ACE) is related to the aerobic capacity of athletes and ACTN-3 that is associated with strength, speed, and power as predominant capacities[5,8]. At a physiological level, observing the ACTN-3 and ACE genes and their respective polymorphisms can become fundamental within performance in sport[9]. Genetic factors do not determine an athlete's success, but it is useful to identify them to enhance performance from the genotype[3].

The presence of ACTN-3 in the muscle benefits sports performance, specifically in strength and power, and the absence of the polymorphism of this gene benefit those athletes that participate in endurance events[1]. In collective sports such as soccer, it is found that the combination of homogeneous RR and heterogeneous RX alleles reflects better performance in strength and speed. In contrast, the combination of homogeneous XX alleles show better performance in endurance competitions; this is associated at a physiological level with the function of the contractile apparatus that is generated from the muscle fibres when doing sports[9]. Female young soccer players, who are faster and more powerful, had a high prevalence of ACE DD and ACTN-3 RX genotypes[10]. ACTN-3 has been shown to have higher performance with R alleles within strength and power[9].

ACE is associated with the D allele with greater volume, muscle strength and a higher percentage of muscle fibres[9]. Subjects with ACTN-3 (RR) polymorphism have longer muscle and specific fibres; in this genotype, the anabolic contribution increases muscle mass[8]. In the ECA polymorphism, it is found that the D allele has greater changes at the muscular level (muscle cross-sectional area) and this condition can enhance the performance on isometric and dynamic strength testing[8]. Therefore, the purpose of this study was to explore the potential relationship between ACTN-3 and ACE gene expression over the change in muscle mechanical and functional properties in youth handballers through a congested tournament.

Material and method

Participants

Thirty players (15 men and 15 women) belong to first division handball teams that participated in a national tournament of I division organised by the Costa Rican Handball Federation. The average age of the 30 participants was 19.3 years (± 3.6) and with an average number of hours of weekly training of 7.5. Those players who participated >45min of an official competition match were included. All participants provided written informed consent for participation in this study, according to the criteria of the Declaration of Helsinki by the World Medical Association[11].

Tournament

A total of 12 matches took place over three consecutive days (Saturday, Sunday, and Monday). Four matches were held each day (two for men and two for women). Each team played one match per day. The pitch had the official measurements (20x40 m) and the matches had 60 minutes of duration (30 minutes each period of play).

Collection and treatment of the genetic sample (DNA epithelial cells)

A mouth rinse was performed with a 5% sucrose solution for 60 seconds, and each rinse was collected in a 15 ml centrifuge tube. Subsequently, 3 ml of TNE [17 mM Tris / HCl (pH 8.0), 50 mM NaCl and 7 mM EDTA] diluted in 66% Ethanol prepared previously in the laboratory were added, the rinsing solution was divided into four tubes, one was used as a rapid test, and the others were kept refrigerated for 2, 15, and 30 days respectively[12].

The DNA was left in isolation, and its respective purification was carried out: using the DNA extraction kit, DNA Blood and Tissue Mini kit (Qiagen, West Sussex, UK), according to the manufacturer's instructions. The purity level of the extracted DNA was analysed using a Nanodrop and an agarose gel 1% to confirm its integrity. PCR and PCR RFLP were used respectively to detect the alleles of the ACE (rs1799752) and ACTN3 (rs1815739) genes. Specific primers were used for the identification of the polymorphisms[13]. To perform the enzymatic digestion of the ACTN3 gene, the enzyme DdeI from the manufacturer Thermo Scientific was used. The product´s reaction was visualized by electrophoresis on agarose gel at 3%, buffer TBE 0,5%; stained with Gel Red (Biotum).

Mechanical muscle properties (tensiomyography)

Before and after each match, rectus femoral muscle contractile responses of the dominant leg were assessed through a tensiomyography (TMG) (TMG, Ljubljana, Slovenia). For this, each participant took a supine position on a portable massage table. The knee joint was fixed in a comfortable extension on a padded pillow where the participants remained relaxed. Two electrodes (5 x 5 cm) (TheraTrode, TheraSigma, Orange, CA, United States) were used, placed at 5 cm from each other on the point of the maximal radial circumference of the muscle. A stimulator (TMG-S2 doo, Ljubljana, Slovenia) induced a quadrangular, single-phase, and 1ms wave of pulse duration between 0.1 s and 110 mA and a precise digital displacement transducer (40 GK, Panoptik doo, Ljubljana, Slovenia) positioned perpendicular to the measurement point of muscle was used this had a spring constant of 0.17 Nmm21 and registered muscle contraction time (TC) and delay time (TD), both expressed in milliseconds (ms), and, maximum radial muscle displacement (DM) expressed in millimetres.

The stimulus was started by inducing muscle contraction, beginning with 40 mA and increasing by 20 mA; a 10-second rest separated the electrical stimuli to avoid post-tetanic activation[14].

Functional muscle properties (Countermovement jump)

This test was used to assess the power of the players. An Axon Jump platform (Bioingeniería Deportiva, San Martín, Argentina) was used with special software (Smart Axon 4.02). Before and after each match, the participants were placed on the platform with their legs shoulder-width apart and their hands on the waist. When prompted, 15 seconds of consecutive explosive jumps were performed. To record the Rate of Perceived Effort (RPE), the Borg scale was used, where 6 corresponds to "very, very light" effort and 20 as "maximum" effort[15].

Statistical analysis

Using a statistical software (SPSS v23, SPSS Inc., Chicago, USA), descriptive frequency analyses (absolute and relative values) were performed to determine the distribution of polymorphisms in the ACTN-3 and ACE genes. Then, a one-way analysis of variance of independent groups was performed to compare the mechanical and functional muscle responses between the polymorphisms expressed for each gene. Bonferroni's post hoc test was considered to determine specific differences. Also, it was calculated the percentages of the changes of each variable analyzed between pre- and post-match. $p< 0.050$ was the level significance selected for all analyses

Results

Table 1 shows the percentage frequencies of gene expression in which it is shown that the polymorphisms most expressed in handball players are ACTN-3 R-X (56.7%) and ACE I-D (43.3%).

There were no neuromuscular differences between groups of polymorphisms in the ACE gene in the variables of the dominant rectus femoris TC (F= 0.113, p= 0.893), TD (F= 0.116, p= 0.891), DM (F= 0.709, p= 0.501), and CMJ (F= 0.825, p= 0.893). Likewise, no differences were found in TC (F= 1.832, p= 0.236), TD (F= 0.205, p= 0.816), DM (F= 1.352, p= 0.276) or CMJ (F= 1.522, p= 0.236) in the ACTN-3 gene (Table 2).

There were no variations in mechanical functions after an official match according to the expressed polymorphism. in the variables according to polymorphism expressed in the ACTN-3 gene: TC (F= 3.358, p= 0.05), TD (F= 1.064, p= 0.359), DM (F= 1.772, p= 0.189), and CMJ (F= 0.827, p= 0.448). No significant differences were found in the ECA gene: TC (F= 0.114, p = 0.892), TD (F= 0.658, p= 0.526), DM (F= 1.216, p= 0.312), and CMJ (F= 0.490, p= 0.618) (Table 3).

Table 1. Frequency and percentage of expression of polymorphisms in ACTN-3 and ECA genes.

	R/R	R/X	X/X	I/I	I/D D/D
ACTN-3	10 (33%)	17 (56.7%)	3 (10%)		
ACE			11 (36.7%)	13 (43.3%)	6 (20%)

Table 2. Muscle mechanical and functional differences according to polymorphism expressed in ACTN-3 and ACE.

	ACTN-3			ACE		
	R/R	R/X	X/X	I/I	I/D	D/D
TC (ms)	25.9 ± 2.9	25.6 ± 3.7	21.8 ± 2.4	25.9 ± 4.1	24.4 ± 2.3	26.1 ± 4.3
TD (ms)	22.4 ± 1.7	22.8 ± 1.8	22.2 ± 1.5	22.6 ± 1.1	22.4 ± 1.7	22.9 ± 2.6
DM (mm)	7 ± 1.8	7.4 ± 1.6	5.8 ± 0.3	7 ± 1.9	7.3 ± 1.5	7.1 ± 1.6
CMJ (cm)	28.3 ± 5.5	32.4 ± 6.1	31.4 ± 6.3	30.2 ± 6	31.4 ± 6.2	31.2 ± 6.6

TC: contraction time; TD: delay time; DM: maximum radial muscle displacement; CMJ: countermovement jump; ms: milliseconds; mm: millimetres; cm: centimetres.

Table 3. Percentage of change in post-match of muscular mechanical and functional variables according to polymorphism expressed in ACTN-3 and ACE.

	ACTN-3			ACE		
	R/R	R/X	X/X	I/I	I/D	D/D
TC (ms)	11.2 ± 13.8	0.2 ± 9.1	1.9 ± 7.5	2.7 ± 13.2	4.7 ± 9.7	5.0 ± 14.4
TD (ms)	1.8 ± 9.5	-2.5 ± 6.13	-0.9 ± 7.4	-3.0 ± 6.3	0.4 ± 6.6	-0.2 ± 10.9
DM (mm)	6.9 ± 24.9	1.1 ± 23.1	28.4 ± 16.2	14.5 ± 32.5	1.9 ± 15	-1.8 ± 19.7
CMJ (cm)	92.9 ± 313.1	6.6 ± 8.9	-13.1 ± 5.7	4.3 ± 11.5	71.2 ± 274.2	4.7 ± 15.9

TC: contraction time; TD: delay time; DM: maximum radial muscle displacement; CMJ: countermovement jump; ms: milliseconds; mm: millimetres; cm: centimetres.

Discussion

The purpose of this study was to explore the potential relationship between ACTN-3 and ECA gene expression over the change in mechanical muscle properties in youth handballers through a congested tournament. According to the available evidence, few studies have explored the genetic influence on handball player´s physiology, physical performance, or other critical factors (risk of injury, anthropometrical characteristics). Only two studies were found that reported outcomes related to handballers[5,16]. The expression frequency of polymorphisms of the genes analysed in our study can be observed in table 1. The most expressed polymorphisms were the ACTN-3 R-X (56.7%) and the ECA I-D (43.3%). ACE genotypes frequencies within a group of 27 handball players found 21 players with insertion homozygous (II), five with heterozygous (ID) and one with deletion homozygous (DD)[5]. These results are in line with previous literature suggesting that RX genotype is the most frequent in handballers as Chilean[16].

Handball is an intermittent team sport that requires both the anaerobic and aerobic energy systems[17,18]. The most prevalent gene expression was the ECA I-D, associated with a higher aerobic and endurance capacity. On the other hand, RX alleles have implied a better predisposition to strength, speed, power movement[9]. In both cases, the frequency and influence of these alleles of ACE and ACTN are associated with the physical and physiological demands that characterize handball[17,18].

In other sport disciplines have been observed similar percentages of prevalence of these genes. For example, in Russian and Lithuanian professional athletes such as weightlifters, powerlifters, and throwers, the prevalence of these polymorphisms' expression was between 43% to 51% for ACE I-D and between 45% to 56% for ACTN-3 R-X[13].

ACE and ACTN are associated with physical performance in physical tests[5,8]. Nevertheless, results obtained in the present study did not evidence any significant difference among the genes expression, which coincide with results found in an Italian athletes' group, who did not present a significant correlation between ACE I/D and ACTN3 (R577X) with power capacity[3].

Other research had reported some influence of these genes on the jump capacity. In male soccer players, individuals with ACTN RR usually had better performance in jump tests than individuals with RX and XX[19]. Besides, ACTN RR and RX female soccer players groups showed a higher capacity during a CMJ test of seven continuous jumps[10]. The players with higher predisposition of these genes' expressions used gain muscle mass and strength and the development of a higher number of fibres type II, that can explain the power responses of the athletes[8].

This study included the analysis of tensiomyography responses of the leg's muscles; however, no previous investigations were found that relate muscle mechanical responses with gene expression. It is known that tensiomyography assess muscles contractile properties[20,21]. In female rugby players, a higher stiffness of vastus lateralis muscle was correlated with higher muscle power assessed by Wingate anaerobic test[20]. Likewise, vertical jump performance had shown an association with tensiomyography responses in power and endurance athletes[21].

The analysis of the changes in the tensiomyography responses and vertical jump seem not to show a significant effect on the gene's expression. However, during the young soccer short-congested fixture period, muscle stiffness of biceps femoris and rectus femoris decreased due to cumulative fatigue during the game and throughout the tournament[14]. In team sports such as handball, in which players frequently perform high-intensity activities[17], the manifestation of muscle fatigue can affect physical performance reducing the force, power, aerobics, and anaerobic capacity during the game. Another important aspect described in the literature is the association between ACTN and low muscle injury risk[9].

Authors should highlight some limitations, as the relative low sample size, to analyse and obtain solid conclusions. On the other hand, only two variables associated with physical performance were analysed, this should be extended in future studies. This could have limited the possible correlations or differences between physical capacities with gene expression like have been informed in previous literature.

Conclusion

The handball players have ACE and ACTN; however, a potentiation of effect was not found on the mechanical or functional muscle responses. According to these results, these genes it seems not to influence on these neuromuscular capacities.

More investigations will be needed to explain and understand the real influence of this gene's expression on the physical performance of handball players. It will be crucial to add other facts like nutrition, physical training experience and anthropometric within the future studies to obtain a more complete scenery about this topic. Also, to consider analysing ACE and ACTN together with physical activities during the games, knowing the influence of these genes' expression on the physical performance players when they are competing will help practitioners have a better understanding of the characteristics of this sport.

Conflict of interest

The authors do not declare a conflict of interest.

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