R577X OF THE ACTN3 GENE AS PREDICTOR OF PHYSICAL PERFORMANCE IN ULTRAMARATHON RUNNERS

R577X DO GENE ACTN3 COMO PREDITOR DE DESEMPENHO FÍSICO EM ULTRAMARATONISTAS

R577X DEL GEN ACTN3 COMO PREDICTOR DE DESEMPEÑO FÍSICO EN ULTRAMARATONISTAS

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ABSTRACT

Introduction: Genetic factors appear to explain why some athletes perform better in competition and training than their peers. Objective: To determine the occurrence of R577X polymorphism of the ACTN3 gene in mountain runners. Methods: The sample consisted of 19 female mountain runners with a mean age of 41.2 ± 6.1 years. Genotyping of R577X polymorphism of the ACTN3 gene was performed by the polymerase chain reaction (PCR) method with DNA extracted from saliva. The genotypic and allelic frequencies of the athletes were evaluated and compared with data from the literature. Hardy-Weinberg equilibrium and Chi-square with Yates correction were used, with a significance level of $p<0.05$. Results: The genotypic distributions did not show any significant differences between the athletes and the control group, with RR = 15.8%, RX = 57.9%, XX = 26.3%. In regard to allelic distribution, the nonfunctional allele was higher in the study group than in the control group, with R = 44.7%, X = 55.3% for $p = 0.0350$. Conclusion: The data revealed a possible relationship between the ACTN3 X allele and athletic performance in Brazilian female mountain runners. **_Level of evidence II; Development of diagnostic criteria in consecutive patients (with "gold" reference standard applied)._**

Keywords: Athletes; Actinin/genetics; Athletic performance; Ethnic groups/genetics.

RESUMO

Introdução: O componente genético parece explicar a melhor adaptação à competição e ao treinamento de alguns atletas com relação a seus pares. Objetivo: Determinar a ocorrência do polimorfismo R577X do gene ACTN3 em atletas corredoras de montanha. Métodos: A amostra foi composta por 19 corredoras de montanha com média de idade de 41,2 ± 6,1 anos. A genotipagem do polimorfismo R577X do gene ACTN3 foi realizada pelo método de reação em cadeia de polimerase (PCR) com DNA extraído da saliva. As frequências genotípica e alélica das atletas foram avaliadas e comparadas com dados da literatura: Equilíbrio de Hardy-Weinberg, Qui-quadrado com correção de Yates, sendo adotado como nível de significância p<0,05. Resultados: As distribuições genotípicas RR = 15,8%, RX = 57,9%, XX = 26,3% em comparação com o grupo controle e as atletas da presente investigação, não mostraram diferença significativa. Quanto à distribuição alélica, o alelo não funcional no grupo estudado com relação ao controle foi maior R = 44,7%, X = 55,3% para p = 0,0350. Conclusão: Os dados revelaram uma possível relação entre o alelo X do ACTN3 e a condição atlética em corredoras de montanha brasileiras. **Nível de evidência II; Desenvolvimento de critérios diagnósticos em pacientes consecutivos (com padrão de referência "ouro" aplicado).**

Descritores: _Atletas; Actinina/genética; Desempenho atlético; Grupos étnicos/genética._

RESUMEN

Introducción: El componente genético parece explicar la mejor adaptación a la competición y al entrenamiento de algunos atletas con relación a sus pares. Objetivo: Determinar la ocurrencia del polimorfismo R577X del gen ACTN3 en atletas corredoras de montaña. Métodos: La muestra fue compuesta por 19 corredoras de montaña con promedio de edad de 41,2 ± 6,1 años. El genotipado del polimorfismo R577X del gen ACTN3 fue realizado a través del método de reacción en cadena de polimerasa (PCR), con ADN extraído de la saliva. Las frecuencias genotípica y alélica de las atletas fueron evaluadas y comparadas con datos de la literatura: Equilibrio de Hardy-Weinberg, Chi-cuadrado con corrección de Yates, siendo adoptado como nivel de significancia p<0,05. Resultados: Las distribuciones genotípicas RR = 15,8%, RX = 57,9%, XX = 26,3% en comparación con el grupo control y las atletas de la presente investigación, no mostraron diferencia significativa. En referencia a la distribución alélica, el alelo no funcional en el grupo estudiado con relación al control fue mayor R = 44,7%, X = 55,3% para p = 0,0350. Conclusión: Los datos revelaron una posible relación entre el alelo X del ACTN3 y la condición atlética en corredoras de montaña brasileñas. **_Nivel de Evidencia II; Desarrollo de criterios diagnósticos en pacientes consecutivos (con estándar de referencia "oro" aplicado)._**

Descriptores: _Atletas; Actinina/genética; Rendimiento atlético; Grupos étnicos/genética._

INTRODUCTION

The improvement of physical activity in high-performance sports may be linked to genetic predisposition.[1] For more than a decade, studies have shown that genetics may explain the different responses and better performance of some athletes in specific modalities when comparing them with their peers.[2] Moreover, genetic factors can influence up to 50% the phenotypic characteristics related to performance, training and physical fitness of high-performance athletes.[3]

As many genes and gene areas have already been related to phenotypes of human physical performance, the association between genetics and phenotypic profile can help athletes developing ideal morphophysiological features for certain sports, making them less susceptible to injuries and more prepared for training and competitions.[1]

Thus, the study of genetic polymorphisms can be a prognosis of physical performance.[4] However, when dealing with studies that consider ethnicity and race of the population studied, we must remember that the phenotypic effects of some polymorphisms may be expressed in multiple ways in different communities.[3] For Ahmetov et al.[1] and Yang et al.[5], genetic polymorphisms associated with sportive success are those that bring benefits, especially related to endurance, sprint and muscle power. In this sense, we highlight R577X polymorphism of the alpha-actinin 3 gene (ACTN3), investigated here because of its association with activities that require muscle strength, sprint, and endurance.[6,7]

Located on chromosome 11q13-q14, this polymorphism is the product of the switch from cytosine to thymine at position 1747 of exon 16, which results in the substitution of arginine (R allele) for a premature stop codon (X allele) at amino acid 577, and thus allowing three genotypes: RR, RX and XX.[8] The switch of arginine or R allele represents the natural transcription of the α-actinin-3 protein in the skeletal muscle, while the stop codon/X allele prevents homozygous individuals (genotype XX) from producing α-actinin-3, decreasing the transverse sections area in muscles composed of type 2 fiber predominance, and thus reducing muscle mass, when compared to RR and RX genotypes.[8,9]

However, the non-expression of α-actinin-3 seems to improve aerobic metabolism, increasing the muscle and cardiorespiratory endurance.[9,10] The relation of ACTN3 with high performance in sports is described by studies that report a greater presence of the X allele in athletes with muscular endurance[11,12], while those with muscle power and sprint have the R allele.[7,12] Considering this, our research aims at better understanding the interaction of ACTN3 R577X polymorphism with the physical performance of female mountain runners in Brazil, since such genetic variation can improve their physical fitness. Moreover, this is an unprecedented analysis of these type of athletes, with the objective of determining the occurrence of *R577X* polymorphism in mountain runners.

MATERIALS AND METHODS

This is a descriptive transversal study carried out according to Resolution 466/12, during the *Ultramaratona dos Perdidos SkyMarathon®*, a 45-km race with 2,900 m of ascent, relative height of 5,800 m, in the second week of July 2017, in Tijucas do Sul, Paraná, Brazil. The study was also approved by a Research Ethics Committee (CEP) under opinion 1,572,571.

The inclusion criteria were runners with experience of three to four years in mountain races, and in at least two races above 21 km and one above 42 km, between 2015 and 2016, who trained five to six times a week, one to two hours a day and more than three hours at weekends, and have not reported musculoskeletal diseases. Runners who did not complete the race within a limit time of 11 hours and who did not signed the Informed Consent Form (IFC) were excluded.

Sample

Of the 34 participants, 15 did not participate in the study because they did not meet the inclusion criteria; thus, the sample consisted of 19 mountain runners, with mean age of 41.2 ± 6.1 years.

Procedures

The runners selected were evaluated in relation to the presence of ACTN3 R577X polymorphism according to the following procedures:

Saliva collection

Saliva collection was performed in the field, in a specific area to support athletes, and the participants selected for the study sat in a chair with their feet supported. Initially, a 3% glucose solution was introduced in the participants' mouths, and they swished the product for two minutes. After this, they spat the liquid in a plastic cup. Then the researcher gently rubbed their jugal mucosa with a wooden spatula and washed the spatula in the cup where they spat the solution, and transferred the entire content to a 15ml Falcon tube.

After collection, the samples were taken in a polystyrene foam box with ice plates to the Laboratory of Genetics and Molecular Biology of a Private Higher Education Institution, in Curitiba/PR, where they were centrifuged at 3000 rpm for 10 minutes. After centrifugation, the supernatant liquid was discarded. We kept the precipitate, added 1300 µL of cell extraction buffer (TRIS 10mM, EDTA 5mM, SDS 0.5%, pH=8) and frozen it at −20 °C[13].

DNA extraction

After defrosting the saliva samples, we added 10 µL of Proteinase K (BioLabs, New England) in each extraction tube and kept them at 65 °C in water bath overnight.

After taking the samples from the water bath, they were gently agitated and transferred to an 2 mL Eppendorf (microtube). The researchers added 500 µL of ammonium acetate (8mM acetate, 1mM EDTA) and mixed the solution in a vortex for five minutes. They were then centrifuged at 13,000 rpm for 16 minutes and separated into two 1.5 mL microtubes (900 µL in each one), disregarding the pellet deposited at the bottom of the tube and adding 540 µL of isopropanol. We gently reversed the tube 20 times to see the DNA.

The content with DNA was centrifuged at 13,000 rpm for seven minutes, the isopropanol was discarded, and we added 1 mL of 70% ethanol. The solution was centrifuged again at 13,000rpm for seven minutes (the supernatant was discarded), and kept at room temperature for drying. The DNA was resuspended at approximately 50 µL of TE (TRIS 10 mM, 1 mM EDTA, pH=7.76) and kept at room temperature for 24 hours. After this period, the samples were stored for two days in the refrigerator and stored at −20 °C.

ACTN3 R577X genotyping

The genotyping of ACTN3 alleles (RR, RX and XX) was performed by the RFLP-PCR technique (polymerase chain reaction through restriction fragment length polymorphism analysis). For this genotyping, we used specific primers (INVITROGEN, Waltham, Massachusetts, USA), 5'-CTGTT-GCCTGTGGTAAGTGGG-3' and 5'-TGGTCACAGTATGCAGGAGGG-3'[14], and the reactions were performed with a final volume of 10 µL in an microtube, containing 1 µL (50 ng) of DNA, 0.5 µL (10 pmol) of each specific primerr, 0.5 of Taqpolymerase (INVITROGEN, Waltham, Massachusetts, USA) and the required amount of SuperMix (INVITROGEN, Waltham, Massachusetts, USA) to complete the final volume.

The PCRs were carried out in a thermal cycler (Biocycler®) (New York, USA), with the following amplification program: a) 95 ºC for 5 min.; b) 94 ºC for 30 sec.; c) 58 ºC for 30 sec.; d) 72 ºC for 30 sec.; e) repeating 30 times the steps b to d; f) 72 ºC for 5 min (final extension).

After amplification, 10 µL of the PCR product was submitted to digestion with 10 units of DdeI restriction enzyme (SIGMA) and incubated for 4 hours in water bath at 37 °C.

Electrophoresis in agarose gel

For analyzing the ACTN3 genotypes, we conducted agarose gel (3%) electrophoresis, colored with ethidium bromide and visualized with an UV transilluminator.

The R577X alleles were distinguished by the presence (577X) or absence (577R) of a restriction DdeI. enzyme site.[14] The ACTN3 R577 variant generates fragments of 205 and 86 base pairs, while the ACTN3 577X allele, of 108, 97 and 86 base pairs.[5] (Figure 1)

Statistical Analysis

The Pearson's Chi-square test was used to compare the frequencies of genotypes with other studies; when the incidence number of some genotype was less than five, we used the Fisher's exact test. The associations between the alleles frequencies were analyzed by 2×2 contingency tables, and by the Chi-square test with Yates correction. The Hardy-Weinberg equilibrium was used to verify the distribution of ACTN3 genotypes. The support tool for tests *was IBM SPSS Statistics* 20 (New York, USA), except for the Hardy-Weinberg Law, which was verified with BioEstat version 5.3 (Belém, Pará, Brazil). All analyses had significance level of p<0.05.

RESULTS

The study included 19 ultramarathoners, with mean age of 41.2 ± 6.1 years, total body mass of 56.9 ± 7.4 kg, height of 163 ± 7.3 cm and fat percentage of 13.73 ± 1.72%. The distribution of the ACTN3 genotype followed the Hardy-Weinberg equilibrium (p=0.45), which shows that the genotypic frequencies found were not different from the values observed in the general population. Therefore, it had no genetic influence on the sample of our study. There were no significant differences in the distribution of ACTN3 genotypes (p>0.05) when compared to athletes from other studies. (Tabela 1)

Regarding the allelic and absolute distribution of ACTN3, no significant differences were found (p=0.51), when compared with the studies by Shang et al.[15] (p=0.64), Belli et al.[16] (p=0.25), Herbert et al.[17] (p=0.23), Ben-Zaken etal.[12] (p=0.79). However, when comparing the results with the research by Coelho et al.[18], there was a significant difference (p=0.03), showing that the X allele is higher for the study sample. (Table 2)

DISCUSSION

Regarding the ACTN3 R577X polymorphism in Brazilian athletes, we verified that, until now, there are no studies focused on this interaction in female mountain ultramarathoners, which reveals the unprecedented character of our study.
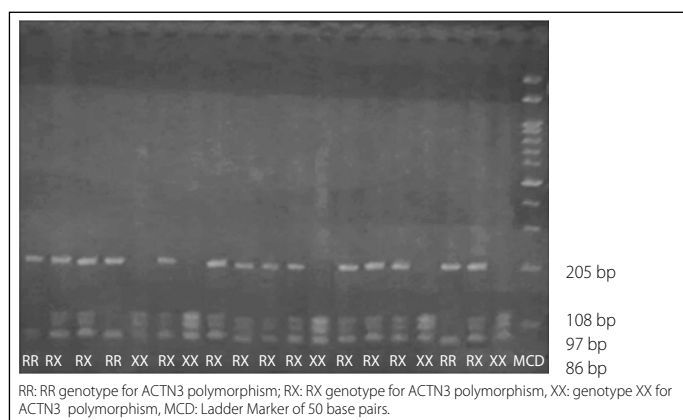

RR: RR genotype for ACTN3 polymorphism; RX: RX genotype for ACTN3 polymorphism, XX: genotype XX for ACTN3 polymorphism, MCD: Ladder Marker of 50 base pairs.

Figure 1.

Table 1. Distribution of genotypic profiles of the 19 ultramarathon athletes in relation to athletes with similar characteristics and control group.

Research (year)	Sample group (n)	RR (%)	RX (%)	XX (%)	*p-value
Current research (2018)	Mountain ultramarathoners (19)	3 (15.8)	11 (57.9)	5 (26.3)	0.06
Shang et al.[15]	Endurance (118)	22 (18.6)	71 (60.2)	25 (21.2)	0.86
Belli et al.[16]	Ultra-endurance (20)	7 (35)	9 (45)	4 (20)	0.38
Herbert et al.[17]	Marathoners (484)	141 (29.1)	245 (50.6)	98 (20.2)	0.43
Ben-Zaken et al.[12]	Endurance (65)	13 (20)	29 (44.6)	23 (35.4)	0.59
Coelho et al.[18]	Control (100)	40 (40)	46 (46)	14 (14)	0.10

*= Chi-square with Yates correction.

Table 2. Allelic distribution of the 19 ultramarathoners in relation to athletes with similar characteristics and with the control group.

Research (year)	Sample group (n)	R (%)	X (%)	*p-value
Current research (2018)	Mountain ultra-marathoners (19)	17 (44.7)	21 (55.3)	0,5164
Shang et al.[15]	Endurance (118)	115 (48.7)	121 (51.3)	0,6476
Belli et al.[16]	Ultra-endurance (20)	23 (57.5)	17 (42.5)	0.2596
Herbert et al.[17]	Marathoners (484)	527 (54.4)	441 (45.6)	0,2389
Ben-Zaken et al.[12]	Endurance (65)	55 (42.3)	75 (57.7)	0,7901
Coelho et al. [18]	Control (100)	126 (63)	74 (37)	0,0350*

*= Chi-square with Yates correction.

The results obtained in terms of absolute and relative ACTN3 genotypic frequency showed that, when compared with studies conducted with endurance athletes from China[15] and Israel,[12] ultra-endurance runners from Brazil,[16] European marathoners[17] and with the Brazilian population in general,[18] there is no statistically significant difference.

Although the literature[12,19,20,15] evidences the genotypic association of ACTN3 with athletic performance in high-performance modalities, this study did not find any connection of genotype with athletic performance, corroborating the studies conducted by Ahmetov et al.,[4] Lucia et al.[21], and Muniesa,[22] who researched the ACTN3 association and the athletic performance of elite Russian rowing athletes, elite European cyclists and European runners, respectively.

On the other hand, studies conducted by Ben-Zaken et al.,[12] Druzhevskaya et al.,[19] Gómez-Gallego et al.[20] showed a relationship between genotype and performance. Ben-Zaken et al.[12] showed evidence of the relationship between genotype XX and the fitness of endurance athletes. Druzhevskaya et al.[19] found that 3.4% of elite athletes focused on muscle strength expressed genotype XX, and Gómez-Gallego et al.[20] observed that RR and RX genotypes are related to the peak power of endurance cyclists.

When comparing the allelic distribution with the control group,[18] we noticed that our sample has a frequency of the higher X allele (55.3% vs 37%), which is in agreement with Shang et al.[15] According to these authors, women have higher frequency of genotype XX (21.2% vs 15.8%), as well as of the X allele (51.3% vs 41.1%), when compared to the control group; but the same is not valid for men, which suggests that the non-functional allele of the gene may provide advantages for women in endurance tests.

Most long-distance athletes have a higher distribution of the X allele,[12] which influences muscle function in fast fibers, directing muscle metabolism to an aerobic pathway, which results in improved endurance performance.[9,10]

Although Yvert et al.[23] point out the R allele presence in elite endurance athletes, and other inconclusive studies have not linked physical performance with ACTN3 R577X polymorphism in athletes with high strength and power[20] and muscle endurance,[6] some studies[11,12] suggest

that the non-functional allele (X) is present in individuals focused on sprint/power modalities, while the mutant allele (R) is more present in muscle endurance athletes.[11,24]

allele, distributed in heterozygous and/or homozygous pattern, which supports the hypothesis that the non-functional allele of ACTN3 is associated with a good performance in high-level sports in Brazilian women who are mountain ultramarathoners.

CONCLUSION

This study was the first to analyze ACTN3 in Brazilian female ultramarathoners and found that 84.2% of the sample had the non-functional X

All authors declare no potential conflict of interest related to this article

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