ASSOCIATION OF ACTN3 R577X AND ACE I/D POLYMORPHISMS IN BRAZILIANS WRESTLERS

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ASSOCIAÇÃO DOS POLIMORFISMOS ACTN3 R577X E ACE I/D EM LUTADORES BRASILEIROS ASOCIACIÓN DE LOS POLIMORFISMOS ACTN3 R577X Y ACE I/D EN LUCHADORES BRASILEÑOS

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ABSTRACT

Introduction: By associating genetics and sport, it is possible to identify subjects with greater capacity to adapt to training, and lower chances of injury. Objective: The investigation evaluated the genotypic and allelic distribution of ACTN3 R577X and ACE I/D polymorphisms in Brazilian high-performance athletes in wrestling and percussion combat sports. Methods: The study included 37 male athletes ranked from first to third place in world scenarios, divided into two groups: wrestling (23 wrestlers, being 11 of Judo, 4 of Greco-Roman style, 8 of Brazilian Jiu Jitsu, with mean age of 27.3 ± 6.9 years) and percussion combat sports (14 athletes with a mean age of 25.7 ± 4.4 years, being 6 of Karate, 3 of Muay Thai, 4 of Taekwondo, 1 Boxing). Genotyping of ACTN3 and ACE I/D polymorphisms was performed by polymerase chain reaction (PCR) from the genomic DNA. Genotypic and allelic distributions were compared with control populations and athletes by Chi-square test and Fisher's exact test; all analyzes considered $p \le 0.05$. Results: The genotypic distributions and allelic frequencies of ACTN3 RR=46%, RX=38% and XX=16%; R=65% and X=35%, and ACE I/D DD=47.7%, ID=34.3% and Il=20%; D=62.9% and I=37.1% did not differ from the control population; however, when compared with wrestling athletes a significant difference was observed. Conclusion: These results suggest an association of ACTN3 R577X and ACE I/D genes with Brazilian high-performance wrestling athletes.

Keywords: athletes; actinin/genetics; peptidyl-dipeptidase A; ethnic groups/genetics.

RESUMO

Introdução: Ao associar genética e esporte, existe a possibilidade de identificar sujeitos com maior capacidade de adaptação ao treinamento com menores chances de lesões. Objetivo: A investigação avaliou a distribuição genotípica e alélica dos polimorfismos ACTN3 R577X e ACE I/D em lutadores de domínio e percussão de alto rendimento brasileiros. Métodos: Participaram do estudo 37 atletas do sexo masculino, colocados de primeiro a terceiro lugar nos cenários mundiais, divididos em dois grupos: domínio (23 lutadores sendo 11 de judô, 4 de luta greco-romana, 8 de jiu-jitsu brasileiro; a média de idade foi 27,3 \pm 6,9 anos) e percussão (14 atletas com média de idade de 25,7 \pm 4,4 anos sendo 6 de caratê, 3 de muay thai, 4 de taekwondo, 1 de boxe). A genotipagem dos polimorfismos do ACTN3 e ACE I/D foi realizada por reação em cadeia da polimerase (PCR) a partir do DNA genômico. As distribuições genotípicas e alélicas foram comparadas com populações controle e de atletas pelos testes do qui-quadrado e exato de Fisher; todas as análises consideraram $p \leq 0,05$. Resultados: As distribuições genotípicas e frequências alélicas do ACTN3 RR=46%, RX=38% e XX=16%; R=65% e X=35% e ACE I/D DD=47,7%, ID=34,3% e II=20%; D=62,9% e I=37,1% não diferiram da população controle, porém, quando comparadas com atletas de luta, constatou-se diferença significativa. Conclusão: Esses resultados sugerem uma associação dos genes ACTN3 R577X e ACE I/D aos lutadores de domínio de alto rendimento brasileiros.

Descritores: atletas; actinina/genética; peptidil dipeptidase A; grupos étnicos/genética.

RESUMEN

Introducción: Al asociar genética y deporte, existe la posibilidad de identificar sujetos con mayor capacidad de adaptación a los entrenamientos con menores posibilidades de lesión. Objetivo: La investigación evaluó la distribución genotípica y alélica de los polimorfismos ACTN3 R577X y ACE I/D en luchadores brasileños de alto rendimiento de técnicas de agarre y golpeo. Métodos: Participaron en el estudio 37 atletas del sexo masculino, colocados de primer a tercer lugar en escenarios mundiales, divididos en dos grupos: agarre (23 luchadores, siendo 11 de judo, 4 de lucha grecorromana y 8 de jiu-jitsu; el promedio de edad fue de 27,3 \pm 6,9 años) y golpeo (14 atletas con promedio de edad de 25,7 \pm 4,4 años, siendo 6 de karate, 3 de muay-thai, 4 de taekwondo y 1 de boxeo). El genotipado de los polimorfismos de ACTN3 y ACE I/D se llevó a cabo por reacción en cadena de la polimerasa (PCR) a partir de ADN genómico. Las distribuciones genotípicas y alélicas fueron comparadas con poblaciones control y atletas por las pruebas del chi-cuadrado y exacta de Fisher; todos los análisis consideraron p \leq 0,05. Resultados: Las distribuciones de los genotipos y frecuencias alélicas de ACTN3 RR=46%, RX=38% y XX=16%; R=65% y X=35% y ACE I/D DD=47,7%, ID=34,3% e Il=20%; D=62,9% e I=37,1% no difirieron de la población control, sin embargo, cuando comparadas con los atletas de lucha, se constató diferencia significativa. Conclusión: Estos resultados sugieren una asociación de los genes ACTN3 R577X y ACE I/D a los luchadores brasileños de alto rendimiento de técnicas de agarre.

Descriptores: atletas; actinina/genética; peptidil-dipeptidasa A; grupos étnicos/genética.

INTRODUCTION

Although the triad training, proper nutrition and psychological factors can contribute to the athletic success¹, it is observed over the years that such factors are not enough to characterize a phenotype of status of physical performance in humans². The sports of combat can be divided into grappling (judo, Brazilian Jiu-Jitsu, Greco-Roman wrestling) and percussion (as Karate, Taekwondo, Muay Thay, boxing, etc)³⁻⁷. But when the dynamics of these modalities are analyzed, the grappling fights present these characteristics of effort: pause of 2:1 or 3:1³⁻⁵ and the fights of percussion with relations of effort: pause of 1:6 to 1:9^{6.7}. It is observed that they are formed by short and intermittent stimulus, being the time of stimulus superior to the time of the pause⁸.

Therefore, athletes of grappling and percussion fights need to have an efficient glycolytic metabolism for energy transfer. This because many times these fights calls for movements of high power to perform muscular actions⁹, and they also need an aerobic capacity adequate to sustain the performance during the fighting 10. Thus, it is suggested that the ability of some wrestlers to protrude in their specific sports of combat can be directly related to genetic predisposition¹¹. Studies carried out in the post-genome era, about genetics and physical qualities have shown the reliability of genetic markers for molecular prognosis of human physical performance¹². There is growing evidence that there are strong genetic influences on athletic performance and for an evolutionary change in performance characteristics for activities of speed, strength and resistance¹³. This phenomenon can be illustrated by the variation R577X in ACTN3 gene, encoding the protein α-actinina-3, a protein that is necessary to perform explosive muscle actions. This protein is almost exclusively expressed in muscle fibers of fast contraction¹⁴ and the I/D polymorphism of the ACE in which the I allele is reported as the most frequent in endurance athletes, while the D allele is present in athletes of strength and muscle explosion¹⁵. So, the objective of this research was to evaluate the genotypic and allele distribution of ACTN3 R577X and ACE I/D polymorphisms in Brazilian grappling and percussion wrestlers of high performance.

METHODS

The population of this study consisted of 37 Brazilian athletes of high performance, with a prominent position on the world scenario in their respective modalities. The selection criterion was athletes with the first to third colocation. This restricts significantly our study group. This study group was divided into two groups: grappling (23 male athletes of high performance being 11 Judo athletes, 4 of Greco Roman Wrestling and 8 Brazilian Jiu-Jitsu athletes, all of them with mean age 27.3 \pm 6.9 years) and percussion (14 male athletes with an average age of 25.7 \pm 4.4 years, being 6 Karate athletes, 3 Muay Thai wrestlers, 4 Taekwondo athletes and 1 of Boxing).

Wrestlers and high-performance percussion athletes with national or international results aged between 18 and 44 years were included in this experiment. The following athletes were excluded: the ones that expressed their intention not to participate in the study; blood samples that for technical reasons were not possible to extract the DNA and amplify it. For this purpose 37 athletes were tested for ACTN3 gene and 35 subjects were tested for ACE gene. The loss of this sample for the ACE gene happened because of non-amplification of two samples. All participants were informed about the research procedures, requirements, benefits and risks before providing written informed consent. The study was approved by the Ethics in Research Committee, of the Faculty Don Bosco PR / BR on the opinion n° 489.086.

For the analysis of genotypes for polymorphisms in genes ACTN3 R577X and ACE I/D, 10 ml of blood were collected from forearm veins of all athletes. The genomic locus containing the polymorphic site was amplified using the PCR-RFLP (polymerase chain reaction associated

with the restriction fragment length polymorphism). The exon 15 of the ACTN3 gene, where the polymorphism was amplified using the following primers: forward 5'-CTGTTGCCTGTGGTAAGTGGG-3' and reverse 5'-TGGT-CACAGTATGCAGGAGGG-3', anchored in the intronic sequence upstream¹⁴.

The reaction system had a total volume of 25 uL, comprising 1x Tag Buffer, 1.5 mM MgCl₂, 0.2 mM dNTPs, 1.0 M of each primer, 1 unit of Tag DNA polymerase and 100 ng genomic DNA as template. The amplification program consisted of the following steps: 95°C for 5 minutes initial denaturation and enzyme release, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds. final extension at 72°C for 30 seconds. On completion of the 30 cycles, there was a 5 minute final extension at 72°C. After amplification, 10 µL of the PCR product were digested by 10 units of Ddel enzyme for 4 hours in a water bath at 37°C. The R or X alleles (codons TGA and CGA) were distinguished by the presence (577X) or absence (577R) of restriction enzyme Ddel site of the $(5-TNA \downarrow C G-3)^{14}$. The restriction fragments were separated by agarose gel electrophoresis at 3%, and revealed with ethidium bromide at 5 ug/ ml. The ACTN3 577R allele generates fragments of 205 and 86 base pairs (bp), while the ACTN3 allele 577X generates fragments of 108.97 and 86 bp¹³.

The I/D polymorphism of the ACE gene is the absence (deletion or Dallele) or presence (insertion and I allele) of 287 base pairs in intron 16. Thus, the 16 intron was amplified using the following primers: (forward 5`CTGGAGAGC-CACTCCCATCCTTTCT'3 and reverse 5'GATGTGGCCATCACATTCGTCAGAT'3)16. The reaction system had a total volume of 25 uL, being composed of 1X Tag Buffer, 3.0 mM MgCl2, 0.2 mM dNTPs, 1.0 M of each primer, 5% dimethyl sulfoxide (DMSO), 1 Tag DNA polymerase and unit 100 ng of genomic DNA as template. The amplification program consisted of the following steps: 95°C for 5 minutes initial denaturation and enzyme release, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 57°C for 1 minute, extension at 72°C for 1 minute. After the completion of the 30 cycles, there was a 5 minute final extension at 72° C. The fragments were separated by agarose gel electrophoresis on 3% and revealed with ethidium bromide at 5 µg/ml. The D allele of the ACE gene produces a fragment of 191 base pairs, while the I allele generates a fragment of 478 base pairs, containing the insert of 287 bp.

According to the literature, the erroneous classification of heterozygous D/I as being homozygotes D/D may occur due to preferential amplification of the D allele and to the inefficient amplification the I allele¹⁷. Therefore, to increase the specificity of the genotyping, the samples that presented D/D genotype were re-evaluated by a new PCR using a specific primer for the incorporation: forward 5'TTTGAGACGGAGTCTCGCTC'3, reverse 5'GATGTGGCCATCACATTCGTCAGAT'3 and the reverse primer 5'-3'. Additional reactions (specific to insert) had the same concentrations of the first PCR reagents. The amplification program was: 95°C for 5 minutes initial denaturation and enzyme release, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 56°C for 1 minute, extension at 72°C for 1 minute. On completion of the 35 cycles, there was a 5 minute final extension at 72°C17.

The results were visualized after electrophoresis on agarose gel and stained with 3% ethicium bromide at $5\mu g/ml$. The appearance of a 408 base pair band is indicative of the presence of I allele, which means that, the samples previously genotyped as D/D are now classified as I/D. Samples classified as I/D and I/I in the first reaction were used as positive controls for the specific reaction for insertion.

Statistical analysis

In order to compare the frequencies of genotypes with other published studies, the chi-square test of Pearson was conducted. When the number of incidence of some genotype was less than 5, the Fisher's exact test was made. The associations between the frequencies of the alleles were verified by 2x2 contingency tables analyzed by Chi-square test with Yates correction. The genotype distribution of the ACTN3

gene and ACE I/D were made by the Hardy-Weinberg equilibrium test. For this purpose we used the BioState 5.0 2007 software, with a significance level of p=0.05

RESULTS

The genotypic frequency of ACTN3 R577X and ACE I/D polymorphisms between all of the wrestlers and the percussion group followed the Hardy-Weinberg equilibrim (ACTN3: $x^2=1.07$ and 0.83, p=0.30 and 0.36, ACE: $x^2=2.47$ and 0.03, p=0.11 and 0.85), but this did not occur with the domain group (ACTN3: $x^2=4.15$, p=0.04; CEA: $x^2=4.87$, p=0.02). Genotype distribution for ACTN3 R577X polymorphism is presented in Table 1. There was no significant difference in genotypic distribution for the ACTN3 R577X polymorphism considering all wrestlers and when these were separate in grappling and percussion groups and the control Coelho¹⁸ $p\ge0.05$. However, when comparing the grappling group of this research with the study of Kikuchi et al. 19 and Rodriguez-Romo et al. 20 it was found a significant difference p=0.0483 and p=0.0066 respectively.

The allele frequency of ACTN3 R577X polymorphism is presented in Table 2. There was no significant difference in allele frequency for the ACTN3 R577X polymorphism among all wrestlers and when they were separated into groups of wrestlers and the control Coelho¹⁸ p \geq 0.05. However, when all the wrestlers and the grappling group of the present study are compared with Rodriguez-Romo et al.²⁰ results, a significant difference of p=0.0225 and p=0.0596 was found respectively.

The genotype distribution for the ACE I/D polymorphism is presented in Table 3. There was no significant difference in genotype distribution for the ACE I/D polymorphism among all wrestlers and when these were separated in grappling and percussion groups and Meira-Lima control et al.²¹ p \geq 0.05. However, when all wrestlers and grappling groups and percussion of this research are compared with the study of Cieszczyk et al.²² a significant difference p=0.0032, p=0.0011 and p=0.0581 was found respectively.

The allele frequency for the ACE I/D polymorphism is presented in Table 4. There was no significant difference in allele frequency for the ACE I/D polymorphism among all wrestlers and when they were separated into groups of wrestlers and Meira-Lima et al. 21 control p \geq 0.05. However, when comparing all the wrestlers and the grappling group of this research, with research of Cieszczyk et al. 22 it was found a significant difference of p=0.0085 and p=0.0143 respectively.

Table 1. Frequency distribution of ACTN3 R577X polymorphisms.

	Genotypic distribution n (%)					
Subjects (n)	RR	RX	XX	p value	p value	p value
All the wrestlers (37)	17(46)	14(38)	6(16)	0.6938	0.1207	0.0151*
Grappling (23)	12(52.2)	6(26.1)	5(21.7)	0.2086	0.0483*	0.0066*
Percussion (14)	5(37.7)	8(57.1)	1(7.2)	0.6643	0.4358	0.2701
Coelho ¹⁸ Control	40(40)	46(46)	14(14)			
Kikuchi et al. 19	38(28)	68(50)	29(22)			
Rodriguez- -Romo et al. ²⁰	23(22.3)	60(54.6)	25(23.1)			

Table 2. Allele frequency of ACTN3 R577X polymorphisms.

	Allele freq	uency n(%)			
Subjects (n)	R	Х	p-value	p-value	p- value
All the wrestlers (37)	48(65)	26(35)	0.8860	0.1015	0.0225*
Grappling (23)	30(65.2)	16(34.8)	0.9110	0.1811	0.0596*
Percussion (14)	18(64.2)	10(37.7)	0.9386	0.3638	0.1737
Coelho ¹⁸ Control	126(63)	74(37)			
Kikuchi et al. ¹⁹	144(53)	126(47)			
Rodriguez-Romo et al. ²⁰	104(49.6)	110(50.4)			

Table 3. Genotypic Distribution of ACE I/D polymorphism.

	Genotypic	distributi	on n (%)			
Sujeitos (n)	DD	ID	II	p-value	p-value	p- value
All the wrestlers (35)	16(45.7)	12(34.3)	7(20)	0.2240	0.8191	0.0032*
Grappling (21)	11(52.4)	5(23.8)	5(23.8)	0.0805	0.8774	0.0011*
Percussion (14)	5(37.7)	7(57.1)	2(7.2)	0.8728	0.2658	0.0581*
Meira-Lima et al. ²¹ (control)	104(32.2)	155(48)	64(19.8)			
Kikuchi et al. ¹⁹	68(50.3)	39(28.9)	28(20.8)			
Cieszczyk et al. ²²	2 (7.1)	18(64.3)	8(28.6)			

Table 4. Allele frequency of ACE I/D polymorphism.

	Allele frequ	ency n(%)			
Subjects (n)	D	I	p valor	p valor	p valor
All the wrestlers (37)	44(62.9)	26(37.1)	0.2849	0.8691	0.0085*
Grappling (23)	27(64.3)	15(35.7)	0.3050	0.9468	0.0143*
Percussion (14)	17(64.2)	11(37.7)	0.6366	0.8227	0.0634
Meira-Lima et al. 21	262/62)	283(37)			
Control	363(63)				
Kikuchi et al. ¹⁹	175(53)	95(47)			
Cieszczyk et al. ²²	22 (49.6)	34(50.4)			

DISCUSSION

This study is the first to investigate the genotypic and allele frequency of ACTN3 and ACE genes in high-performance Brazilian grappling and percussion wrestlers. It was observed (in Table 1), a higher frequency of the RR genotype, 52.2%, followed by RX 26.1% and XX 21.7% in the grappling wrestlers of this research. When the same sample was compared to the study made by Kikuchi et al.¹⁹ which evaluated Judo Japanese athletes and Rodriguez-Romo et al.²⁰, who studied Spanish Judo athletes, there was a significant difference between the studies. It is worth mentioning that the RR and RX genotype are related to a better performance in activities requiring speed, muscle strength, hypertrophy and the athletes who have a predominance of type IIx fibers ^{13,14,23-25}.

In the study made by Kikuchi et al.¹⁹, when the genotyping of 135 Japanese Judo athletes was performed, the authors concluded that judo athletes with an international level had a higher correlation with the RR and RX genotype, what demonstrates a genetic predisposition. This genetic predisposition corroborates the present research. However Rodriguez-Romo et al.²⁰ performed the genotyping of 108 athletes and did not found a positive association with the R577X polymorphism and the status of being an elite athlete in judo, at least in the Spanish population. For the same authors, at least according to the ACTN3 R577X polymorphism, the elite judo athlete's genotype looks like the mixed type of athletes and non-athletes with force and muscle power characteristics, demonstrating that sports performance is polygenic.

In the genotype distribution of R and X alleles (Table 2), the present study found a distribution of 65% for the R allele and of 35% for the X allele for all wrestlers who were included in the study. Several works in different modalities, Greeks teenagers and 40 meter race Moran et al.²⁶ finnish athletes of strength and power Niemi and Majamaa²³, sprinters and Spanish road cyclists Fiuza-Luces et al.²⁷ demonstrated an association between allele R with strength and power sports, and X allele with endurance sports. But the data of grappling and percussion wrestlers are somewhat scarce and controversial, Kikuchi et al.¹⁹ found in Japanese athletes an allele distribution of 53% for the Rallele and 47% for allele X, values which corroborate this research. However, Rodriguez-Romo et al.²⁰ investigating Spanish wrestlers had a distribution for the R allele of 49.9% and 50.4% of the X. These findings discard a great influence of performance for this polymorphism. These findings suggest that an individual may possess innate characteristics that benefit him in modalities which have specific characteristics, this may be a result of genetic variation maintained by natural selection, for example, the 577X allele of the ACTN3 gene²⁸.

Regarding the genotype distribution of ACE gene (Table 3), a higher frequency of the DD genotype was observed: 52.4% followed by 23.8% for ID and II in the grappling group of the present research. These values present a significantly different between genotype distributions compared to Cieszczyk et al.²² who studied Polish and Lithuanian judo athletes. Recent studies have shown that the I allele is more frequent in endurance athletes, while the D allele is in athletes with strength and muscle explosion²⁹. Studying Japanese elite athletes, Kikuchi et al.¹⁹ reported values of 50.3%, 28.9% and 20.8% for the DD, ID and II genotypes respectively, showing an association between the wrestlers of this study and the D allele, a fact that has to reinforces the present research. However, the study of Cieszczyk et al.²² with 28 elite wrestlers of Poland and Lithuania, the authors found a genotype distribution of 7.1%, 64.3% and 28.6% for DD, ID and II respectively, values that are different from those from this research.

About the allele distribution of the ACE I/D gene (Table 4), the present investigation found values for the D allele of 62.9%, 64.3% and 64.2% and 37.1% of the I, 35,7% and 37.7% for all wrestlers, grappling and percussion groups, respectively. When these data were compared with the research of Cieszczyk et al.²² a significant difference was found, the authors reported conflicting data with the present investigation, where the distribution of the D allele was 39.3% and the I allele of 60.7% to the authors, the highest incidence of genotype I/II is due to the length of a fight that can reach 5 min. In a population of Japanese elite wrestlers, Kikuchi et al.¹⁹ found an allele distribution of 63% for D and 37.7% for the I allele, values close to those observed in the present study, demonstrating a genetic predisposition to the studied sample.

A possible hypothesis for the favoring of this sample for the studied genes and their alleles linked to the strength and speed, and the other

studies, could reside in the sustaining of epigenetics, which considers that the practice of physical exercise on a regular basis and searches the over compensation, answer this influenced by genes and life experiences. Therefore the changes not stable, but communicable, interact during a biological response, and that the interaction between genes and stimuli can change in much the expected results³⁰.

CONCLUSIONS

In conclusion the results presented here suggest a genetic predisposition for Brazilian elite fighters which were included in this research. It was verified that the genotypic distribution of Brazilian fighters of this research, were favorable to activities of strength and power, because they were larger than in the control population. ACTN3 - RR (46% vs 40 %) and ACE - DD (45.7% vs 32.2%). The current research also suggests that the more predisposed to grappling and percussion fights, are individuals with the distribution of alleles of genes ACTN3 and ACE more significant regarding to the strength and muscle power. This because these fights are characterized by short and intermittent stimuli, being the time of stimulus superior than that of the pause. However, further studies should be performed to clarify the possible role of ACTN3, ACE and other polymorphisms to determine the athletic performance the elite fighters. The results can be used in the identification and selection of young athletes of fighters that have a greater genetic potential favorable to succeed in sports to combat of grappling and percussion in the design of customized training based on genotype.

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

AUTHOR CONTRIBUTIONS: Each author made significant individual contributions to this manuscript. MRR (0000-0002-2979-7282)*, ZCON (0000-0001-8579-9664)*, FS (0000-0002-6555-7227)* and PF (0000-0001-9877-1257)* contributed to the collection of data and writing of the manuscript. FS (0000-0002-6555-7227)*, OM (0000-0002-6555-7227)*, OM (0000-0002-5422-9623) and JCB (0000-0001-5729-9582)* followed the data collections, guided the construction of the study, and assisted in the statistical analysis and revision. All authors contributed to the intellectual concept of the study. *ORCID (Open Researcher and Contributor ID).

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