

Association of genetic polymorphisms with physical capacities and body composition in older women

Associação de polimorfismos genéticos com capacidades físicas e composição corporal em idosas

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Abstract – The elderly population has grown substantially, and the decline in physical capacities and increase in the body fat percentage are important characteristics of aging. Genetic factors may explain these declines and studies related to this issue are justified because they predict what physical capacities present larger declines in different individuals and enable the adoption of strategies to slow them. Thus, the aim of this study was to evaluate the effect of ACE I / D and ACTN3 R / X genetic polymorphisms on body fat, muscle strength and power levels, aerobic capacity, flexibility and agility in older women. Sixty-six older women were genotyped with respect to ACTN3 and ACE polymorphisms for the division of groups and submitted to anthropometric measurements, physical tests in the AAHPERD and RIKLI and JONES test batteries and IPAQ to determine the level of physical activity and the Food Consumption Marker Form. Older women with XX genotype in relation to ACTN3 genotype had lower levels of flexibility of upper and lower limbs and lower cardiorespiratory fitness. Moreover, in relation to the ACE genotype, ID individuals exhibited higher cardiorespiratory fitness and lower body fat percentages. In relation to the other variables, there was no statistical difference among groups. It was concluded that the genetic variants under study play a role in some of the physical capacities and body composition in elderly women. In the future, data of this nature will enable each individual to have specific health interventions directed to the variables showing higher genetic potential for decline.

Key words: Physical fitness; Aging; Strength.

Resumo – A população idosa tem crescido de forma substancial e o declínio nas capacidades físicas, além do aumento na porcentagem de gordura corpórea, são características importantes do envelhecimento. Fatores genéticos podem explicar estes declínios e pesquisas relacionadas a essa temática se justificam porque predizer quais capacidades físicas apresentarão maiores declínios em cada indivíduo possibilita a adoção de estratégias para retardá-los. Assim objetivamos avaliar o efeito dos polimorfismos genéticos ECA I/D e ACTN3 R/X nos níveis de gordura corporal, força e potência muscular, capacidade aeróbia, flexibilidade e agilidade em idosas. 66 idosas foram genotipadas em relação aos polimorfismos da ACTN3 e da ECA para divisão dos grupos e submetidas a medidas antropométricas, testes físicos da bateria de testes da AAHPERD e RIKLI E JONES, IPAQ para determinar o nível de atividade física e o Formulário de Marcadores de Consumo Alimentar. Mulheres idosas com o genótipo XX em relação ao gene da ACTN3 apresentaram menores níveis de flexibilidade dos membros superiores e menor capacidade cardiorrespiratória. Por outro lado, em relação ao gene da ECA, os indivíduos ID apresentaram maior capacidade cardiorrespiratória e menor porcentagem de gordura. Em relação às outras variáveis não houve diferença estatística entre os grupos. Concluímos que as variantes genéticas estudadas têm influência em algumas das capacidades físicas e na composição corporal em idosas. No futuro dados desta natureza possibilitarão cada indivíduo ter suas intervenções em saúde direcionadas às variáveis que ele apresenta maior potencial genético para declínio.

Palavras-chave: Aptidão física; Envelhecimento; Força.

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INTRODUCTION

Aging is the phase of physiological and functional performance decline after an improvement during childhood, reaching maximum levels from late adolescence to the age of 30 years¹, being a result of genetic and environmental factors such as level of physical activity, eating habits, smoking, environment in which the individual is inserted, among others². Regardless of the exact factors that lead to aging, it is known that it results in sensory, cognition and physical fitness loss³.

Genetic polymorphisms (variations of a DNA sequence that affect more than 1% of the population⁴, which can occur due to base exchange, duplication, insertion or deletion of a gene⁵) in the genes of the angiotensin converting enzyme (ACE) and alpha-actinin protein 3 (ACTN3), which can potentially influence physical fitness in the elderly. ACE plays a role in the renin angiotensin system (an important physiological pathway in the cardiovascular system, fluids and electrolyte balance), converting angiotensin I into angiotensin II, which in turn plays a vasoconstrictor role in cell proliferation and aldosterone secretion of adrenal glands⁶. Polymorphisms related to ACE are insertion (allele I) or deletion (allele D), which play a role in increasing (allele D) or decreasing (allele I) enzyme activity⁷. Allele I of ACE gene seems to be associated with the best predisposition of the aerobic capacity, as well as with response to training. This may be due to increased energy availability resulting from improvement in cardiac output and capillarization of the skeletal muscle, improved use of energy substrate due to increased stocks of intramuscular fatty acids, alteration in mitochondrial density and increased muscle myoglobin⁸. Allele D seems to be associated with increased predisposition to gain in muscle strength due to neural adaptations and greater hypertrophy resulting from the local action of the enzyme⁷. ACTN3 is located in the Z-line inside muscle cells, where it helps to anchor actin filaments⁹. R577X polymorphism in the ACTN3 gene is classified as nonsense, in which there is an exchange of C à T nucleotide at position 1747 of the exon¹⁶, resulting in the exchange of the triplet of nucleotides responsible for the translation of arginine – then becoming a stop codon or triplet, generating a truncated protein. Individuals who have the XX genotype have lack of ACTN3, while heterozygotes (RX genotype) have fewer amount of ACTN3, but no disease¹⁰. ACTN3 is found in fast-twitch fibers (type II), suggesting that R577X polymorphism is related to functionality, power and muscle strength¹¹, as shown by Kim et al.¹², who found lower frequency of XX genotype for the ACTN3 gene in elite athletes compared with non-athletes and also by Norman et al.¹³, who showed that individuals with XX genotype for the ACTN3 gene have lower signaling for metabolic pathways associated with hypertrophy and less use of muscle glycogen during exercise. Thus, those who express ACTN3 (RX or RR genotypes) have advantage in activities that require strength or muscle power, while those who do not express ACTN3 (XX genotype) have advantage in predominantly aerobic activities.

The elderly population is growing worldwide and also in Brazil, and there are very few surveys seeking to associate genetic variations in ACE and ACTN3 to physical fitness in the elderly¹⁴⁻¹⁵, especially in the Brazilian population¹⁶⁻¹⁷. Studies on this issue are primarily justified for showing the influence of genetics on physical fitness in the elderly in order to identify the loss of physical fitness before the individual gets old, which can harm ordinary activities of the daily living and provide data that can justify and contribute to future studies aimed at determining whether genetic polymorphisms may influence the magnitude of the response to interventions with exercise. Thus, the aim of this study was to evaluate the effect of ACE I / D and ACTN3 R / X genetic polymorphisms on body fat, muscle strength and power levels, aerobic capacity, flexibility and agility in older women.

METHODOLOGICAL PROCEDURES

Sample

Women enrolled in physical education programs for the elderly were invited to participate in this cross-sectional study (pre-training evaluation). After detailing of all procedures and doubts were cleared, those interested signed the Informed Consent Form and before inclusion in the study, participants were submitted to health screening in order to exclude volunteers who had health conditions that would contraindicate carrying out the tests or inability to perform them: musculoskeletal disorders, presence of symptoms suggestive of cardiovascular disease without medical supervision and presence of cardiovascular disease and / or risk factors for such diseases without having performed maximal exercise testing with ECG. This study was approved by the Ethics Research Committee of the Faculty of Philosophy Sciences and Languages of Ribeirão Preto - FFCLRP (University of São Paulo USP). Data collection occurred with 66 older women, who were submitted to the following evaluations, in addition to those related to physical fitness: body mass and height measurement used a scale (graduation of 50g) with stadiometer (precision of 1.0 mm) (Welmy W200ALCD), body fat percentage used bioimpedance (Maltron BF-906)¹⁸, level of physical activity used the International Physical Activity Questionnaire (IPAQ) (interview that assesses frequency in days and duration in minutes of activities performed for more than ten minutes continuously in a normal week, classified as intense, moderate and walking)¹⁹ and assessment of the nutritional status by the Food Consumption Marker Form (indicates the frequency of consumption of 10 food groups in the days of a normal week)²⁰. Although not included in the objectives, these two instruments were used because their results can interfere in the relations of genetic variations with body fat percentage and physical fitness - the other variables are important to characterize the sample.

Physical fitness assessment

Aerobic capacity was evaluated using the six-minute walk test (distance

traveled on a rectangle route measuring 4.57 m x 18.28 m), flexibility through tests to reach behind the back (distance between the distal ends the two middle fingers) and the sit and reach test (sitting in a chair, the distance between the distal end of the middle finger of the hand and the distal end of the third toe is measured), and in both tests the individual chooses the side of body to carry out the exercise, considering the best of two attempts. Strength was evaluated by the extension and elbow flexion test (highest number of repetitions for 30 seconds with a dumbbell of 2.27 kg)²¹ and agility was evaluated by agility and dynamic balance test of AAHPERD (American Alliance for Health, Physical Education, Recreation and Dance). The test begins with volunteer sitting on a chair, then moving to the right or left (start by participant's preference side) towards a cone positioned 1.5 m behind the chair and 1.8 m to the side of the chair, going back and sitting on the chair in order to remove the feet from the ground and walk to the opposite side. Two cycles were performed and the shortest time in seconds of two attempts was recorded²².

Genotyping

Peripheral blood sample was collected and the DNA was extracted using the phenol-chloroform method²³ (Sigma-Aldrich, Brazil). Then, ACE I / D (rs1799752) polymorphisms were amplified by polymerase chain reaction (PCR) and the resulting PCR products were genotyped using agarose gel electrophoresis. Primers of the Exxtend brand (Brazil) used were F-5'-CTGGAGACCACTCCCATCCTTTCT-3' and 5'-R-GAT-GTGGCCATCACATTCGTCAGAT-3'¹⁵. Fragments without insertion (allele D) and with insertion (allele I) of 190 and 490 bp, respectively, were detected in 1.5% agarose gel containing SYBR Green (Sigma-Aldrich, Brazil). For ACTN3 R577X (rs1815739) polymorphisms, a fragment of 291 bp was amplified with the following primers: 5'-CTGTTGCCTGTGG-TAAGTGGG-F-3' and 5'-R-TGGTCACAGTATGCAGGAGGG-3'¹⁵. Subsequently, amplicons were digested with enzyme Dde I for 17h at 37°C. R577X variation resulted in fragments of 108, 97 and 86 bp. Digestion of the R577 allele resulted in fragments of 205 and 86 bp, and digestion of 577X allele resulted in fragments of 108, 97 and 86 bp. The fragments were detected in 3% agarose gel containing SYBR Green¹⁵.

Statistical analysis

Study participants were divided into different groups, corresponding to each of the genetic polymorphisms. Subsequently, it was examined whether the different dependent variables have normal distribution (Kolmogorov-Smirnov test) and the variances of the different groups are similar (Levene test). As data met these conditions, one-way analysis of variance (ANOVA) was used for comparison between the different ACE I / D polymorphisms (groups ID, II and DD) and Student's t test for independent samples among different ACTN3 R / X polymorphisms (groups XX and RX / RR). Whenever necessary, the post hoc Tukey test was used, and the significance level was 5%.

RESULTS

Table 1 shows data regarding age, height, body mass (BM), body mass index (BMI) and body fat percentage. Both in relation to ACTN3 gene as in relation to ACE gene, there was no significant differences among groups for all variables, except for group ID for the ACE gene, which showed lower body fat values compared to the other two groups [$F_{(2, 60)} 4.741$; $p=0.0123$].

Table 1. Characteristics of the sample in terms of mean, standard error and sample size.

Groups (n)	ACTN3			ACE	
	XX (n)	RX/RR (n)	DD (n)	ID (n)	II (n)
Age (years)	63.9 ± 2.9 (13)	60.1 ± 1.1 (52)	63.2 ± 1.8 (26)	61.2 ± 1.8 (23)	59.2 ± 1.9 (16)
Height (m)	1.60 ± 0.01 (13)	1.60 ± 0.01 (52)	1.59 ± 0.01 (26)	1.60 ± 0.01 (23)	1.60 ± 0.02 (16)
BM (Kg)	72.7 ± 5.5 (13)	70.4 ± 1.2 (52)	69.6 ± 1.8 (26)	67.3 ± 2.8 (23)	78.2 ± 5.8 (16)
BMI (Kg/m ²)	28.3 ± 1.9 (13)	27.5 ± 0.7 (51)	27.4 ± 0.6 (26)	26.0 ± 0.9 (23)	30.0 ± 2.0 (16)
BF%	31.8 ± 3.7 (12)	31.3 ± 1.4 (51)	33.4 ± 1.5 (25)	26.6 ± 2.1 (23)*	35.6 ± 3.1 (15)

BMI: body mass index; BM: body mass; BF%: Body fat percentage; *: $P < 0.05$ versus II and DD.

Table 2 shows the results for tests that assess physical fitness (agility, flexibility of lower limbs, aerobic capacity, flexibility and strength of upper limbs). There was difference among groups for aerobic capacity [$t_{(60)} 2.412$; $p=0.0190$] and flexibility of upper limbs [$t_{(60)} 2.558$; $p=0.0131$] for ACTN3 gene - group XX showed lower performance in the six-minute walk test and in the test of achieving the hands behind the back when compared to group RX / RR. For the ACE gene, difference was found between ID group compared to the aerobic capacity of group II, with group ID showing better results [$F_{(2,59)} 5.098$; $p=0.0091$].

Table 2. Tests that assess physical fitness described as mean, standard error and sample size.

Groups (n)	ACTN3			ACE	
	XX (n)	RX/RR (n)	DD (n)	ID (n)	II (n)
Agility (sec)	24.7 ± 3.2 (12)	23.0 ± 1.2 (51)	20.8 ± 2.1 (51)	22.8 ± 1.2 (23)	26.0 ± 2.9 (15)
Sit and reach (cm)	- 1.5 ± 3.1 (15)	1.2 ± 1.2 (51)	1.0 ± 1.6 (24)	0.6 ± 1.9 (23)	0.3 ± 2.8 (15)
6 min (m)	502 ± 31 (12)	565 ± 11 § (50)	541 ± 13 (25)	592 ± 18 (23) #	509 ± 25 (14)
Reaching behind back (cm)	- 13.9 ± 5.6 (11)	- 3.3 ± 1.5 § (51)	- 4.4 ± 2.1 (24)	1.8 ± 2.4 (23)	11.6 ± 4.5 (15)
Elbow flexion and extension (repetitions)	17.4 ± 1.2 (12)	19.6 ± 0.7 (52)	18.4 ± 0.8 (26)	20.4 ± 1.1 (23)	18.7 ± 1.2 (15)

§ $p < 0.05$ versus XX; #: $P < 0.05$ versus II.

Regarding the IPAQ - short version, the results for the level of physical activity and time spent sitting during the week and during the weekend are presented in Table 3. No statistical difference for any of the two genes was found.

Table 3. IPAQ values - short version described as mean, standard error and sample size.

Group (n)	ACTN3			ACE	
	XX (n)	RX/RR (n)	DD (n)	ID (n)	II (n)
Walking (min/week)	353 ± 147 (12)	260 ± 30 (52)	212 ± 43 (26)	335 ± 82 (23)	294 ± 50 (15)
Moderate PA (min/week)	423 ± 153 (12)	439 ± 61 (52)	327 ± 75 (26)	523 ± 94 (23)	492 ± 143 (15)
Intense PA (min/week)	100 ± 68 (12)	133 ± 38 (52)	100 ± 53 (26)	204 ± 66 (23)	56 ± 33 (15)
Time spent sitting during week (min/day)	197 ± 40 (12)	223 ± 22 (52)	198 ± 34 (26)	270 ± 33 (22)	206 ± 25 (14)
Time spent sitting during weekend (min/day)	280 ± 39 (12)	244 ± 23 (52)	266 ± 34 (25)	283 ± 34 (23)	206 ± 23 (14)

PA: physical activity; min / week: minutes per week; min / day: minutes per day.

Table 4 shows the results for the nutritional status of individuals. For the ACTN3 gene, there were differences among groups in the consumption of soft drinks [$t_{(54)} 2.269$; $p=0.0273$]. The ACE gene showed difference between groups ID and DD in the consumption of vegetables and cooked vegetables [$F_{(2,53)} 6.454$; $p=0.0031$] and sausages [$F_{(2,53)} 3.965$; $p=0.0249$].

Table 4. Consumption of foods (days / week) described as mean values, standard error and sample size.

Group (n)	ACTN3			ACE	
	XX (n)	RX/RR (n)	DD (n)	ID (n)	II (n)
Raw salad (days/week)	5.0 ± 0.8 (11)	5.3 ± 0.3 (45)	4.4 ± 0.5 (20)	6.2 ± 0.4 (22)	4.9 ± 0.8 (14)
Vegetables and cooked vegetables (days/week)	5.5 ± 0.7 (11)	4.8 ± 0.3 (45)	3.6 ± 0.5 (20)	5.9 ± 0.3 (22) §	5.3 ± 0.7 (14)
Fruits (days/week)	6.5 ± 0.3 (11)	5.7 ± 0.3 (45)	5.2 ± 0.5 (20)	6.2 ± 0.4 (22)	6.2 ± 0.6 (14)
Beans (days/week)	4.9 ± 0.7 (11)	4.4 ± 0.4 (45)	4.9 ± 0.6 (20)	5.0 ± 0.5 (22)	3.0 ± 0.8 (14)
Milk or yogurt (days/week)	5.1 ± 0.8 (11)	4.9 ± 0.4 (45)	5.0 ± 0.5 (20)	5.2 ± 0.5 (22)	4.4 ± 0.8 (14)
Fried foods (days/week)	1.5 ± 0.7 (11)	0.6 ± 0.2 (45)	0.6 ± 0.2 (20)	1.1 ± 0.5 (22)	0.6 ± 0.3 (14)
Sausage (days/week)	1.8 ± 0.7 (11)	0.9 ± 0.2 (45)	0.3 ± 0.1 (20)	1.7 ± 0.5 (22) §	1.3 ± 0.4 (14)
Cookies (days/week)	2.2 ± 0.8 (11)	1.1 ± 0.2 (45)	1.3 ± 0.4 (20)	1.5 ± 0.4 (22)	1.0 ± 0.5 (14)
Sweets (days/week)	1.4 ± 0.7 (11)	1.6 ± 0.3 (45)	1.8 ± 0.6 (20)	1.4 ± 0.4 (22)	1.6 ± 0.6 (14)
Soft drinks (days/week)	2.0 ± 0.8 (11)	0.7 ± 0.2 (45) §	0.9 ± 0.3 (20)	0.9 ± 0.3 (22)	1.4 ± 0.5 (14)

§ $p < 0.05$ versus XX; \$: $P < 0.05$ versus DD.

DISCUSSION

Table 1 presented data on the sample characteristics, in which groups of individuals studied were similar, except for group ID for the ACE gene, which showed lower body fat percentage. Literature seems to indicate that allele I is associated with lower body fat percentage, as well as to better use of fatty acids for energy generation⁸. Charbonneau et al.²⁴, in a study of physically inactive older adults ($n = 243$) in the United States, found no difference between genotypes for body fat percentage, only a tendency for lower body fat percentage for genotype II. Although our data are not fully in accordance with literature, we believe that this may be due to the fact that group ID for ACE gene present, although not statistically different, higher levels of physical activity and higher consumption of foods considered “healthy”.

For physical tests, whose data are shown in Table 2, we have chosen to use the RIKLI and JONES test batteries and the agility test and dynamic balance of AAHPERD because these are tests already validated, of low operating cost, practical and with good reproducibility^{22,25}. Group ID of ACE gene showed better aerobic capacity compared to group II. This result is contrary to literature, which indicates genotype II to have a greater tendency to have better aerobic capacity⁸. This was also demonstrated by Hagberg et al.²⁶ in their study with older women in the United States with different levels of physical activity (n = 58). The difference of our results compared to those from literature may be because group ID for the ACE gene is presented as the most physically active group compared to the other two groups for the ACE gene (II and DD).

As for the ACTN3 gene, our results showed better aerobic capacity and flexibility of the upper limbs for group RR / RX, as demonstrated in Table 2. Regarding the aerobic capacity, literature points to a predisposition to better aerobic capacity of allele X of ACTN3 gene in adults^{11,13,27}. However, no studies were found in literature associating flexibility with R577X polymorphism for the ACTN3 gene, as well as studies associating flexibility with aerobic capacity in the elderly. These findings indicate our study as pioneer in the association of these physical abilities with ACE ID and ACTN3 R577X polymorphisms in the elderly.

It is noteworthy that the differences between results found in this study and those of literature may be related to differences in the age group studied, test protocols used, countries of origin and level of physical activity of participants^{13,27}, and in the future it would be interesting to analyze whether there is variation in gene and protein expression of ACE and ACTN3 in different age groups and subjects with different levels of physical activity, despite the difficulty in obtaining the tissues in which these genes are expressed (kidneys and lungs for ACE and skeletal muscles for ACTN3), which would help in the understanding of our results. In addition, the number of individuals in our study, as well as in studies used for discussion, can be considered a limiting factor due to the importance of a large number of subjects in studies with population genetics.

Finally, there was no statistical difference among groups for IPAQ data and little variation among the eating patterns of different groups - only one type of food out of 10 for ACTN3 and two for ACE, being one healthy and the other unhealthy. Even if there is little difference among groups for the level of physical activity and dietary patterns, these data help in the understanding of our results due to its biological value, which reinforces the importance of considering genetics associated with environmental factors.

CONCLUSIONS

Older women with ID genotype in relation to the ACE gene have higher cardiorespiratory fitness and a lower body fat percentage. On the other hand, regarding the ACTN3 gene, individuals from group XX had

lower flexibility of the upper and lower limbs and lower cardiorespiratory capacity.

In literature, the association of physical fitness with ACE ID and ACTN3R577X polymorphisms seems to be well defined in the population of adults and young people, but additional studies are needed for older individuals. In practical terms, results of this nature associated with environmental factors may contribute to indicate which physical capacities have genetic predisposition to be reduced when individuals get older in order to seek to avoid such declines.

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