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**Lack of association between angiotensin-converting enzyme genotype and muscle strength
in Peruvian older people***

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Aging can be associated with decreasing muscle strength, and related factors are comorbidities, sex, physical activity, and possibly genetic factors. Among genetic factors the renin-angiotensin system is of interest, but data on the Peruvian population is lacking. The objective of our study was to evaluate the association of grip strength and angiotensin convertase enzyme (ACE) polymorphism in Peruvian older people. A cross-sectional study in a convenience sample of 104 participants over 60 years in Lima, Perú, with analysis of the ACE polymorphism, was performed. We studied 104 participants, 46 men (44,2%) and 58 women (55,8%), with a mean age and standard deviation (SD) of 73,7 (7,4) years, range between 60–90 years. The frequency of D/D, I/D and I/I genotypes was 12,7; 43,7 and 43,7% respectively. The genotype distribution of ACE polymorphism agreed with the Hardy—Weinberg equilibrium ($p=0,746$). The mean (SD) of grip strength in the D/D, I/D and I/I polymorphisms were 24,8 (7,2); 22,8 (7,2) and 23,4 (7,6) kg respectively; no significant difference was observed ($p=0,41$) between genetic groups. In this small convenience sample of older Peruvians, no association was found between grip strength and ACE genotype.

Key words: muscular strength, aging, polymorphism, angiotensin convertase enzyme, genetic, dynapenia, frailty

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The aging process involves reductions in muscle mass (sarcopenia) and strength (dynapenia) at a rate of 0,5–1% and 2–3% per year, respectively, after the age of 50 years [12]. Sarcopenia is defined as a progressive and generalized skeletal muscle disorder involving the accelerated loss of muscle mass and function and it is associated with adverse outcomes (falls, functional decline, frailty, and mortality) [8]. Dynapenia is the age-associated loss of muscle strength that is not caused by neurologic or muscular diseases, and the most common detection method is via measurement of handgrip strength, and if warranted a test for knee extension strength [7, 17]. The measurement of muscle function in geriatric clinical practice is performed by measuring the grip strength (GS) [2, 21]; it has been shown that a decrease in the strength of grip is related to poor prognostic health outcomes such as disability, falls, fractures and increased mortality [25].

In a recent systematic study on genetic associations with aging muscle, it was found that angiotensin convertase enzyme (ACE) rs1799752 (insertion/deletion) polymorphism is one of the 10 DNA polymorphisms that were significantly associated with muscle phenotypes relevant to sarcopenia and dynapenia [19]. A local renin-angiotensin (SRA) system is involved in skeletal system physiology; the ACE catalyzes the conversion of angiotensin I to angiotensin II, having hypertrophic effects on skeletal muscle [13]. Angiotensin II may contribute to the development of sarcopenia and dynapenia, thus accelerating protein degradation through proteolytic systems (ubiquitin-proteasome), and the depression of protein synthesis by inhibiting growth hormone/factor I insulin-like growth (GH/IGF-I) [22]. The ACE gene is encoded on the long arm of chromosome 17 (17q23), and consists of 26 exons and 25 introns. The presence or absence of a 287bp element in the ECA gene is related to three genotypes: insertion (I/I), insertion/deletion (I/D) and deletion (D/D). Allele studies have reported that allele I is associated with muscular endurance [20], and allele D with muscular strength [1]. There is now a growing interest in establishing the association between polymorphisms of ACE with muscle strength and physical performance in older people, to understand the role of the SRA in the process of sarcopenia and dynapenia and to find a target for future interventions. To date, there have been no studies of those associations in Peruvians. The objective of the present study was to evaluate the association of grip strength (dynapenia) and ACE polymorphism in a sample of older Peruvians.

Materials and methods

An observational and cross-sectional study was carried out. The study was conducted in the Geriatric Day-Clinic at «Hospital Nacional Guillermo Almenara» in Lima, Perú, between January 2016 and December 2018. The research project was approved by the Hospital's Ethics Committee, and all participants signed informed consent. The method of patient selection was

not probabilistic. The people of mestizo ancestry, who entered to the study were part of an investigation on the use of antagonists of angiotensin II, memory performance and its relationship with ECA polymorphisms. The participants were older than 60 years, without a cognitive disorder (Mini-Mental State Examination (MMSE) score >24). Exclusion criteria: participants with depressive symptoms (Geriatric depression scale, short version, <5), patients diagnosed with Parkinson's disease, history of traumatic brain injuries; the presence of visual or auditive impairment; hand osteoarthritis; current medication including antidepressants, antipsychotics, anxiolytics or anticonvulsants.

Variables. Grip strength: To assess muscle strength, a digital hand dynamometer (CAMRY) was used. The participants were asked to be in a sitting position in a chair with both feet on the floor, shoulders adducted and without rotation, forearm and wrist in a neutral position. The best of two GS measures (two in each hand) was selected for the analyses [15].

Up & Go test: Measurement of the time in which the patient from a sitting position, should get up, walk 3 meters, turn and return to the sitting position [18].

Body mass index (BMI): Height and weight of the participants were measured to calculate BMI, defined as weight in kilograms divided by the square of height in meters (kg/m^2).

Calf Circumference: The maximum calf perimeter of the dominant leg was measured in centimeters with the subject standing and the weight distributed in the two legs.

Genomic analysis: After signing the informed consent, blood samples were collected in EDTA tubes of each participant. DNA was extracted from the leukocytes using the standard phenol/chloroform method and amplified by a polymerase chain reaction process. ECA I/D polymorphism (rs1799752) was detected using the method described by Franken et al [9].

Other variables: we obtained information about multimobility and comorbidities [24] (dyslipidemia, diabetes, hypertension, smoke habit, education years, age, gender and Charlson Comorbidity Index scores [6] from clinical charts.

Statistical analysis. Descriptive statistical data were presented as percentage (%), mean with standard deviation (SD) and ranges. Chi-square analysis was performed for the evaluation of deviation from Hardy—Weinberg equilibrium. To analyze the association between GS and ACE I/D genotypes, the mean difference was analyzed. Differences were considered statistically significant if $p < 0,05$. The statistical package SPSS Version 12.0 (SPSS Inc., Chicago, United States) was used to analyze the data.

Results and discussion

104 people over 60 years were included. The study population consisted of 46 (44,2%) men and 58 (55,8%) women, with a mean age and standard deviation (SD) of 73,7 (7,4) years, range between 60–90 years. In the studied population, the frequency of D/D, I/D and I/I

genotypes was 12,7; 43,7 and 43,7% respectively, with allelic frequency D and I of 34,5 and 65,5% respectively. The analysis of the distribution of genotype frequencies was consistent with a population in Hardy—Weinberg equilibrium ($\chi^2=0,1051$; $p=0,746$), *tabl. 1*.

Table 1

Descriptive and bivariate analysis by sex of the sample, n=104

Variables	All sample	Male (n=46)	Female (n=58)	p-value
Age in years, mean (SD*)	73,7 (7,4)	75,80 (3,53)	71,98 (7,66)	0,002
Education years, mean (SD*)	11,7 (3,8)	11,91 (3,53)	11,59 (4,04)	0,7
Charlson Comorbidity Index score, mean (SD*)	0,7 (1,0)	0,91 (1,01)	0,60 (1,03)	0,1
Dyslipidemia n (%)	36 (34,62)	18 (39,13)	18 (31,03)	0,4
Type 2 Diabetes Mellitus n (%)	21 (20,19)	9 (19,57)	12 (20,69)	0,8
Hypertension n (%)	65 (62,50)	32 (69,57)	33 (56,90)	0,2
Smoking habit n (%)	5 (4,85)	2(4,35)	3 (5,26)	0,8
Body Mass Index, mean (SD ¹)	27,74 (4,01)	27,26 (3,73)	28,11 (4,21)	0,3
Barthel index	97,91(4,66)	98,13(3,14)	97,75(5,60)	0,7
Calf circumference (cm), mean (SD*)	35,48 (3,31)	36,27 (2,20)	34,72 (4,01)	0,02
Up & Go test (sec) mean (SD*)	12 (4,22)	11 (3,95)	12,83 (4,37)	0,03
Grip strength (kg), mean (SD*)	23,30 (7,34)	29,0 (6,15)	18,70 (4,42)	<0,001
ACE/ID polymorphism				
I/I n (%)	50 (48,1)	21 (45,65)	29 (50,00)	0,7
I/D n (%)	42 (40,4)	20 (43,48)	22 (37,93)	0,6
D/D n (%)	12 (11,5)	5 (10,87)	7 (12,07)	0,8

*Standard deviation.

The GS mean and SD in the study population distributed according to the frequency of genotypes D/D, I/D and I/I was 24,8 (7,2) kg, 22,8 (7,2) kg and 23,4 (7,6) kg respectively. When we analyzed GS according to sex, the muscle strength in the female sex according to genotype frequency D/D, I/D and I/I was 20,0 (3,8) kg, 17,9 (4,2) kg and 19,0 (4,7) kg respectively. In male participants, GS according to the frequency of genotypes D/D, I/D and I/I was 31,6 (3,8) kg, 28,1 (5,9) kg and 29,2 (6,8) kg respectively. It can be observed that in both sexes the D/D genotype has a greater GS, however, the difference was not statistically significant (*tabl. 2*).

Table 2

Bivariate analysis by D/D vs. I/I ACE polymorphisms, n=104

Variables	D/D, n=12	I/D, n=42	I/I, n=50	ID/II, n=92	p-value, DD vs ID/II
Grip strength (kg), mean (SD*)	24,84 (7,18)	22,75 (7,19)	23,39 (7,59)	23,0 (7,07)	0,4
Female	19,99 (3,79)	17,88 (4,21)	19,03 (4,73)	18,52 (4,50)	0,5

Male	31,64 (3,79)	28,12 (5,84)	29,21 (6,78)	28,68 (6,29)	0,1
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*Standard deviation.

In the study population, no association was found between GS and ECA I/D genotype. It should be noted that it is the first study of its kind in Peru. The results on the influence of the ECA I/D genotype on GS in other populations have variable results, some have found a relationship and others have not. Those who found no relationship between the ECA I/D genotype and muscle strength were in Danish, Korean and Spanish nonagenarians [4, 10, 15]. Studies that have found a relationship between genotype ECA I/D and muscular strength come from Japan [23, 26], Spain [11] and Australia [16], which coincide in the findings of greater GS in the carriers of polymorphism I/D and I/I compared to D/D.

The findings of the present study could be considered compatible with a recent meta-analysis carried out of Genome-Wide Association Studies, who concluded that gait speed, in 31 478 people aged over 60 years of different cohorts in the USA, Europe, and Australia, had no association with genes such as ACE and others like ACTN3, COMT and APOE [3]. It has been explained that the negative findings in these studies can be explained because impairment of muscle strength and gait speed in older people could have other fisiopathologic pathways including central nervous system malfunction, musculoskeletal conditions such as sarcopenia and osteoarthritis, cardiovascular disease, visual function, psychological factors, and other factors. The complexity of the phenotype makes it difficult to show an association [5]. On the other hand, it has been described that muscle strength, balance and gait are subdomains of intrinsic ability. Although genetics is one of the determinants of intrinsic capacity, also the personal and health characteristics of older people contribute significantly to it [14].

Conclusion

We conclude that in a small convenience sample of Peruvians attending a geriatric day hospital, no association was found between GS and ECA I/D genotype. This is the first study of its kind in Peru.

The authors declare that there are no conflicts of interest regarding the publication of this article.

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Отсутствие связи между генотипом ангиотензин-превращающего фермента и мышечной силой у пожилых людей в Перу

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Старение может быть связано с уменьшением мышечной силы, а сопутствующими факторами являются заболевания, пол, физическая активность и, возможно, генетические факторы. Среди генетических факторов представляет интерес ренин-ангиотензиновая система, но данные о перуанской популяции отсутствуют. Целью исследования — оценка связи силы сцепления и полиморфизма ангиотензин-конвертазного фермента (АКФ) у пожилых людей в Перу. Было проведено перекрестное исследование в выборке из 104 участников старше 60 лет в Лиме, Перу с анализом полиморфизма АКФ. Мы изучили 104 участника, 46 (44,2%) мужчин и 58 (55,8%) женщин, со средним возрастом и стандартным отклонением (*SD*) 73,7 (7,4) года, в диапазоне 60–90 лет. Частота генотипов *D/D*, *I/D* и *I/I* составила 12,7; 43,7 и 43,7% соответственно. Распределение полиморфизма АКФ по генотипу соответствовало равновесию Харди—Вайнберга ($p=0,746$). Средняя (*SD*) сила сцепления при *D/D*, *I/D* и *I/I* полиморфизмах составила 24,8 (7,2); 22,8 (7,2) и 23,4 (7,6) кг соответственно. Не выявлено достоверных различий ($p=0,41$) между генетическими группами. В этой небольшой удобной выборке пожилых перуанцев не было обнаружено связи между силой сцепления и генотипом АКФ.

Ключевые слова: мышечная сила, старение, полиморфизм, ангиотензин-конвертазный фермент, динапения, слабость