Study of Association of the *ace2* Polymorphisms rs2285666, rs879922, and rs1978124 with Muscle Strength in Sedentary Young Adult

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Abstract Skeletal muscle is a plastic tissue with contractile capacity to generate force. Muscle mass plays a crucial role in determining muscle strength. However, the diversity of genetic features among individuals can significantly influence muscle mass development. Among these genetic factors are polymorphisms in the *ace* gen which codify for angiotensin-converting enzyme (ACE), an enzyme that belongs to the renin-angiotensin system (RAS). In addition, there are single nucleotide polymorphisms (SNP) of angiotensin-converting enzyme 2 gene (*ace2*), that codify for an enzyme that is part of the non-classical RAS axis, which has recently been shown to prevent atrophic and fibrotic processes in skeletal muscle. However, the influence of *ace2* SNPs on skeletal muscle strength is unknown. This research aims to study the association between the SNPs rs879922, rs2285666, and rs1978124 of the *ace2* gene with isometric muscle strength in sedentary young adults. Thirty-eight healthy sedentary men (18-25 years old) who met the exclusion criteria were recruited for the study. Anthropometric measurements were then taken, and a venous blood sample was extracted to determine the allele of each polymorphism studied. Skeletal muscle strength was measured using a handgrip dynamometer. Our results show no differences in grip strength between alleles of *ace2* polymorphisms nor in comparing the muscle strength between the most common haplotypes. We can conclude that there is no association of rs879922, rs2285666, and rs1978124 *ace2* polymorphisms with isometric grip strength in sedentary young adults, nor is there an association between *ace2* haplotypes and muscle strength.

Keywords: SNPs, Muscle Strength, ACE2, Renin Angiotensin System.

1. INTRODUCTION

Skeletal muscle is a dynamic tissue that adapts to different stimuli and plays a pivotal role in human locomotion and overall mobility [1]. Skeletal muscle is composed of muscle fibers containing multiple myofibrils, in which it is possible to identify several repetitive structures called sarcomere, considered the contractile unit [2]. Due to muscle plasticity, muscle mass can decrease in atrophic conditions causing a loss of strength. On the other hand, muscle mass gain is known as hypertrophy, a process generally associated with increased force generation [3]. It should be noted that several factors can influence the regulation of muscle contraction and the changes in muscle mass. Among them is the renin-angiotensin system (RAS), a hormonal axis that has several functions, some of them associated with cardiovascular and renal systems, such as regulation of blood volume and pressure regulation [4-6]. RAS is composed of two axes: classical and non-classical. The main components of classical RAS include angiotensinogen (AGT), renin, angiotensin I (Ang-I), angiotensin II (Ang-II), and the angiotensin-converting enzyme (ACE). The non-classical RAS includes angiotensin-converting enzyme 2 (ACE-2), Mas receptor, and angiotensin 1-7 (Ang-(1-7)) [6, 7]. Ang-II is converted from Ang-I through the ACE [8]. Most of the effects of Ang-II are mediated through two receptors: angiotensin type-1 receptors (AT1) and angiotensin type-2 receptors (AT2). Ang-II induces

pathological effects through AT1 receptors [9, 10]. Furthermore, several studies have demonstrated that Ang-II harms skeletal muscle tissue. Specifically, it decreases muscle mass and function by reducing protein synthesis and increasing degradation processes [8].

ACE2 is a carboxypeptidase that forms Angiotensin 1-9 (Ang 1-9) from Ang-I [11], which is subsequently cleaved by ACE to generate Ang-(1-7) [12]. This axis can counter most harmful effects of the ACE/Ang-II/AT1 axis, especially in pathological conditions [13]. Various studies demonstrated that Ang-(1-7) through Mas decreases the muscle atrophy produced by Ang-II in mice [14]. In addition, Ang-(1-7) through Mas inhibits the pro-fibrotic effects of TGF-β1, increasing muscle strength, and improving physical performance in mice [15, 16].

On the other hand, it has been described that the classical and non-classical RAS axis may be regulated by the genetic variability of the ACE enzyme since its function is strongly influenced by polymorphisms [17, 18]. DNA polymorphisms can be categorized based on DNA sequence variations between alleles. The two main types are single nucleotide polymorphisms (SNPs), which involve a single base change, and insertion-deletion polymorphisms (indel) [19]. One example of polymorphism is the indel in the ace gene, which consists of the presence (insertion, allele I) or absence (deletion, allele D) of repeated 287-base fragments in intron 16. Individuals can be typed as homozygous (I/I or D/D) or heterozygous (I/D) [20]. ace I/D polymorphisms about genetic variation in human muscular phenotypes have been extensively studied. However, research outcomes have been contradictory [21-23], and the association between ace polymorphisms and muscle strength is controversial [24-29].

The genetic variability of ace2, a gene located on the X chromosome [30], has been recently studied in humans. Specifically, SNPs in the gene codifying this enzyme have been related to the cardiovascular system. No indels have been found in the ace2 gene, so the study of the genetic variability of ace2 is reduced to SNPs [31]. The polymorphisms rs879922, rs2285666, and rs1978124 have been among the SNPs more frequently studied in association studies [32].

Currently, there are no reports of studies that associate ace2 polymorphisms with muscle function, despite that ACE2 is the main enzyme that forms Ang-(1-7), peptide that benefits muscle function and morphological changes. This study evaluates as SNPs in the ace2 gene can be associated to muscle strength.

2. MATERIAL AND METHODS

2.1. Participants

Thirty-eight healthy and sedentary volunteer men from Santiago, Chile, participated in the study (18-25 years old). The characteristics of the participants are shown in (Table 3). Regarding diet, it was observed that 84.21% of individuals use an omnivorous diet, while only 15.79% follow a vegetarian or vegan diet. Among the participants, 78.95% were right-handed and 21.05% were left-handed. The exclusion criteria included diagnosed pathologies that affects the strength of the upper extremity such as carpal tunnel syndrome, fracture of the phalanges, carpal bones, metacarpus, radius, ulna, and humerus, de quervain's tenosynovitis, rotator cuff syndrome. In addition were excluded individuals with alteration in the sensitivity of the hand, diagnosed pathologies of the central nervous system (stroke, parkinson, dementia, diagnosed pathologies of the peripheral nervous system (peripheral neuropathy, neuromuscular dystrophies); diagnosed renal, cardiac, hepatic or pulmonary failure; use of anti-hypertensive drugs, ACE inhibitors, anti-inflammatories drugs or statins, the presence of nonspecific pain in the upper extremity, regular performance of high-demand jobs for the upper extremity, sports activity performed less than three times per week involving the upper extremity, physical or sports activity practiced regularly for less than six months that have affected the upper extremity.

2.2. Study design:

Participants were recruited and enrolled in the study. They were instructed to perform the strength test to familiarize themselves before the evaluation. The following week, they were applied for the isometric grip test through the dynamometer and subjected to blood extraction. A document of informed consent was explained to

individuals and signed for them when accepted to participate. The study was approved by the Metropolitan University of Educational Sciences ethics committee and conducted according to the Helsinki Declaration.

Table 1. Characteristics of the participants

Variable	Media n=38	Standard deviation n=38
Age (y)	21.39	1.87
Body weight (kg)	74.05	12.15
Height (m)	1.73	0.05
Body mass index (kg/m^2)	24.62	3.86

2.3. Grip Strength Test

A 5-position Dynatron® manual grip hydraulic dynamometer was used to measure grip strength of the dominant hand. The individuals underwent testing while seated, maintaining a 90º angle in their elbow flexion. The positioning of the handle was adjusted to suit their hand perfectly. They were instructed to exert maximum force by squeezing the dynamometer for three trials, each separated by a 30-second interval. The data analysis involved the average from these three trials. To avoid inter-rater inconsistency, a single specialist guided and recorded the test. The protocol was carried out as previously described [33]

2.4. Blood Collection and Genetic Analysis

5 ml syringes or the Venoject vacuum system were used to draw venous blood with 21-gauge needles. To store the blood, Vacutainer® tubes were filled with EDTA K2 as an anticoagulant. Genomic DNA was extracted from blood samples through an Axygen brand commercial kit, according to the manufacturer's instructions. The extracted genomic DNA was stored at -20°C until use. Subsequently, its concentration was measured by UV spectrophotometry at 260 nm. The integrity of the genomic DNA was confirmed by DNA electrophoresis in a 0.8% agarose gel in Tris-acetate EDTA (TAE) solution. For the amplification of a DNA segment containing the sequence that includes each of the polymorphisms, the technique of polymerase chain reaction (PCR) was used. For this, three pairs of primers were used (1 pair for each region containing the polymorphisms to be analyzed) (See Table 2).

Table 2. Primers used to detect polymorphism by PCR

Name	Sequence (5'-3')	5'	3'	Product size (pb)
rs1978124-SP rs1978124-ASP	TCTTCCTGGCTCCTTCTCAGC ACCACAATGGCAGAGAAAGGG	6,168 7,211	6,188 7,191	1,043
rs2285666-SP rs2285666-ASP	GTTTGTAACCCAGATAATCC GTTGAAACACACATATCTGC	14,796 14,906	14,815 14,925	129
rs879922-SP rs879922-ASP	TTGTGTTAAGATCTTGTCCC AATAAACTGAGCTCCAGC	34,242 34,407	34,255 34,424	182

The amplified PCR products were visualized in 1.5% or 2.5% agarose gel electrophoresis. Specific restriction enzymes digested the PCR-amplified products to determine the corresponding polymorphism. Thus, to detect the rs1978124 polymorphism, the Sau96 I enzyme was used, rs2285666 with the Alu I enzyme, and rs879922 with the Bfa I enzyme (Table 3).

Table 3. Restriction enzymes used for SNPs detection

Localization	Name	Enzyme	Product size
Intron 1	rs1978124	Sau96I	966-82
Intron 3	rs2285666	AluI	78-52
Intron 11	rs879922	BfaI	146-42

3. RESULTS AND DISCUSSIONS

3.1. Distribution of Strength Values Among Participants

The participants had an average strength of 39.78 ± 5.7 kg. The majority of the participants (15 individuals) showed a strength in the range of 35-40 kg, while ten volunteers had values ranging from 40 to 45 kg (Figure 1). In the extreme ranges, seven people had strength values ranging from 45 to 50 kg, four participants had a strength in the interval 30-35 kg, and only two subjects had values less than or equal to 30 kg (Figure 1).

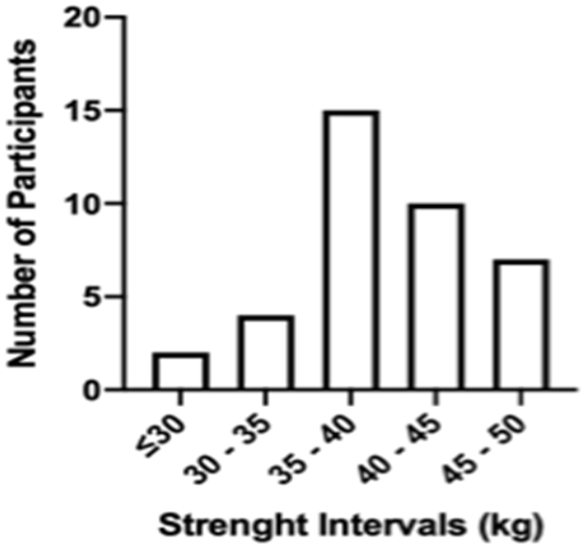

Figure 1. Distribution of grip strength values. The figure shows the number of participants and the strength obtained according to 5 kg intervals.

3.2. Types of Polymorphism and Alleles

Regarding the distribution of the alleles found in the different polymorphisms, Table 4 specifies their frequency and percentages. The C allele is the most abundant (65.79%) in the rs2285666 polymorphism, compared to the T allele (34.21 %). In the case of the SNP rs879922 polymorphism, the C allele showed a higher frequency (84.21 %) when compared to the G allele (15.79 %). Finally, the C allele in the SNP rs1978124 polymorphism presents a higher frequency (78.95 %) than the T allele (21.05 %).

Table 4. Frequencies of the alleles in each of the polymorphisms

Variable	Allele	Frequency	Percentage
SNP rs2285666	C	25	65.79%
	T	13	34.21%
SNP rs879922	C	32	84.21%
	G	6	15.79%
SNP rs1978124	C	30	78.95%
	T	8	21.05%

3.3. ace2 Polymorphisms, Anthropometric Characteristics, And Muscle Strength

The anthropometric values of the subjects separated by allele for the *ace2* rs2285666 polymorphism are shown in

Table 5. A statistically significant difference was only found between the T and C alleles for the height. In the case of the rs879922 and rs1978124 polymorphism, the anthropometric values did not vary significantly between the alleles of both SNPs, as shown in Tables 6 and 7 respectively.

To compare the muscle strength with the polymorphism alleles, the muscle strength of the participant was normalized to their body mass index. Regarding the *ace2* polymorphism rs2285666 alleles, it was found that there are no statistically significant differences between the C and T alleles in terms of muscle strength, nor the case of the C and G alleles in the rs879922 polymorphism, nor the C and T alleles of the rs1978124 polymorphism (Table 8).

Table 5. Anthropometric values of the alleles for SNP rs2285666

Variable	Allele T n=13	Allele C n=25	p-value
Body weight (kg)	76.15 ± 11.93	72.96 ± 12.35	0.450
Height (m)	1.76 ± 0.05	1.72 ± 0.03	0.013*
Body mass index (kg/m²)	24.61 ± 4.1	24.61 ± 3.8	0.997
* p < 0.05			

Table 6. Anthropometric values of the alleles for SNP rs879922

Variable	Allele G n=6	Allele C n=32	p-value
Body weight (kg)	69.66 ± 13.69	74.87 ± 11.89	0.342
Height (m)	1.72 ± 0.05	1.73 ± 0.04	0.512
Body mass index (kg/m²)	24.02 ± 3.65	23.86 ± 4.32	0,421

Table 7. Anthropometric values of the alleles for SNP rs1978124

Variable	Allele C n=30	Allele T n=8	p-value
Body weight (kg)	69.66 ± 13.69	74.87 ± 11.89	0.862
Height (m)	1.72 ± 0.05	1.73 ± 0.04	0.990
Body mass index (kg/m²)	23.43 ± 4.01	24.83 ± 3.85	0.829

Table 8. Grip strength and ace2 polymorphisms

SNPs	Allele	Normalized strength	p-value
rs2285666	T	1.65 ± 0.21	0.852
	C	1.62 ± 0.31	
rs879922	G	1.75 ± 0.27	0.264
	C	1.61 ± 0.28	
rs1978124	C	1.64 ± 0.29	0.783
	T	1.61 ± 0.23	

3.4. ace2 Haplotypes, Anthropomorphic Characteristics, and Muscle Strength

We found four more frequent haplotypes to be analyzed: HAP1 (TCC), HAP2 (CGT), HAP3 (CCC), and HAP4 (CCT). The other haplotypes constituted less than 5 % of the sample, so they were not considered for the analysis. The average anthropometric values of each haplotype are shown in Table 9. No significant differences were found between the groups when comparing the mass, height, and body mass index between the four haplotypes.

Regarding the distribution according to haplotypes, this was 17 people with the CCC alleles (44.74%), 11 with TCC (28.95 %), 4 with CGT (10.53 %), 3 with CCT (7.89 %), 1 with TGC (2.63 %), 1 with TCT (2.63 %) and 1 with CGC (2.63 %).

Muscle strength was also normalized with body mass index to compare the difference between haplotypes. However, muscle strength did not vary significantly between the four haplotype groups (See Table 10).

Table 9. Anthropometric values and ace2 haplotypes

Haplotype	Body weight (kg)	Height (m)	Body mass index (kg/m2)
HAP1 (TCC) n=11	75.27 ± 12.05	1.76 ± 0.06	24.33 ± 4.19
HAP2 (CGT) n=4	66.75 ± 10.87	1.72 ± 0.05	22.43 ± 3.53
HAP3 (CCC) n=17	73.41 ± 10.96	1.71 ± 0.03	24.82 ± 3.48
HAP4 (CCT) n=3	83 ± 19.46	1.73 ± 0.03	27.38 ± 5.63
p-value	0.365	0.174	0.426

Table 10. Grip strength and ace2 haplotypes

Haplotype	Normalized strength	p-value
HAP1 (TCC)	1.64 ± 0.23	0.725
HAP2 (CGT)	1.69 ± 0.28	
HAP3 (CCC)	1.62 ± 0.33	
HAP4 (CCT)	1.45 ± 0.12	

This research indicates no association of the ace2 polymorphisms rs2285666, rs879922, and rs1978124 with isometric muscle strength in sedentary young adults. Additionally, no correlation was found between the different ace2 haplotypes and isometric muscle strength.

The present paper studies the relationship between ace2 polymorphisms with muscle function. In 2010, the expression of ACE2 in skeletal muscle tissue was reported [34]. Before this, its role was only mainly associated with the cardiovascular system and renal tissue [35]. In 2014, cell signaling studies involved ACE2 in anti-fibrotic processes, and its specific relationship with skeletal muscle tissue was reported. In addition, Ang-(1-7), the main product of the ACE2 activity, was also described as an anti-atrophic peptide [14-16].

The three SNPs analyzed in this study are the most frequently reported in association studies of ACE2 in the cardiovascular system [31]. However, our data show no significant differences in the grip strength between the groups categorized by allelic and haplotype distribution. This result may be explained because it has not yet been precisely determined which of the ace2 SNPs (the three studied or another) influence changes in ACE2 enzymatic activity, ace2 gene expression, or transcriptional activity [31]. These roles would finally allow us to determine the behavior of the non-classical RAS axis. For example, if any SNPs directly affect the ACE2 function, it will ultimately influence the axis effect on skeletal muscle under physiology or pathological conditions, such as muscle atrophy and fibrosis. In this sense, it can be speculated that ace2 polymorphisms could affect events such as AKT/PKB phosphorylation by Ang-(1-7) through its Mas receptor, which ultimately reduces muscle atrophy [14]. Also, it can be proposed that the influence of ace2 polymorphisms in inhibiting TGF-β signaling by Ang-(1-7) decreases fibrosis, diminishes total tissue collagen, and increases the force produced by the muscle [15].

In studies of the ace2 rs1978124 polymorphism and its association with cardiac morphology, the T allele was

associated with increased left ventricular mass in Caucasian women with type 2 diabetes mellitus [36]. The G allele of the rs879922 polymorphism was associated with the morphological alteration of heart in subjects with hypertrophic heart disease [37]. These antecedents can be explained because these alleles are possibly involved in an alteration in ACE2 activity. Therefore, increased ACE2 activity increases Ang-(1-7) levels, producing antihypertrophic effects in the heart [38]. This suggests that these SNPs may change ACE2 activity, which could interfere with anatomical processes in the heart and possibly in other tissues, such as skeletal muscle. Even so, more studies are required to clarify which SNPs, or combinations of them, produce significant changes in the enzyme activity that could ultimately affect the role played by the no-classical RAS axis in skeletal muscle. Another alternative is that ace2 polymorphisms indirectly affect skeletal muscle performance due to altered cardiac function.

Under certain pathological conditions such as muscle atrophy or fibrosis, there is an imbalance of the Ang-II/ACE/AT1 and Ang-(1-7)/ACE2/Mas axis. However, if the balance between these axes is restored by inhibiting the classical and activating no-classical, an improvement in muscle function is achieved [39]. This suggestion indicates that possibly the role of ACE2 is essential in individuals with pathologies that affect the musculoskeletal system but not in the case of healthy participants, which could explain the lack of significant differences in the ace2 alleles of the polymorphisms with the grip strength obtained in the present study. It could be interesting to evaluate whether there is a difference in the loss of muscle mass and strength in pathological conditions such as sarcopenia.

It is essential to study possible association of different ace2 polymorphisms with skeletal muscle in people with muscle pathologies. Thus, it is relevant to carry out studies in patients with dystrophies or atrophic processes, conditions where the role of this axis in the musculoskeletal system has been studied, demonstrating that it exerts a relevant regulatory activity. An example is the effect anti-atrophic induced by Ang-(1-7) in a murine model of Ang-II-induced muscle atrophy, or the reduction of fibrosis in limb-girdle muscular dystrophy in rats [14, 15, 39, 40]. Other group of participants interesting to carry out genetic studies are adolescents and older adults, two age ranges in which the muscular system is in conditions of development and aging [41]. Finally, it will be valuable to carry out these genetic studies of association between ace2 polymorphism and muscle strength on people with different physical activity levels, such as the elite athletes, a population where ace polymorphisms have been extensively studied showing an possible association between physical performance and alleles I and D of this enzyme [22, 23].

It would also be engaging in further research to study the ace and ace2 polymorphisms together and associate them with the levels of muscle strength. These enzymes belong to RAS, with opposite functions [13], so it would be pertinent to analyze how several ace and ace2 polymorphisms can affect the activity of these enzymes and thus determine a specific and/or differential muscle phenotype and function. There are also other polymorphisms studied related to muscle strength. One of them is Alpha-Actinin-3 (ACTN3), a component protein of the Z line, and peroxisome proliferator-activated receptor alpha (PPARα). This transcriptional regulator controls the genes responsible for fatty acid oxidation in skeletal muscle [23]. However, studies related to these polymorphisms with the ACE indels [42] have shown significance when analyzed, suggesting that these different polymorphisms, including the ACE2, should be studied together for a complete approach to determining a muscle phenotype.

Therefore, this research shows the study of the association between ace2 polymorphism and muscle strength in young, healthy, sedentary men for the first time. No difference was found, which allows us to visualize future research where other variables can be considered in the experimental groups.

CONCLUSIONS

In conclusion, our study reveals that ace2 polymorphisms, specifically rs2285666, rs879922, and rs1978124, exhibit no significant associations with isometric grip strength in sedentary young adults. Furthermore, we found no compelling relationship between ace2 haplotypes and grip strength in this group. To comprehensively understand the genetic determinants of muscle strength, further investigations involving more extensive and diverse cohorts spanning various health and physical conditions and across different age groups are essential. Exploring concurrent associations between ace and ace2 gene polymorphisms will contribute to a more comprehensive understanding.

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DOI: https://doi.org/10.15379/ijmst.v10i1.2913