

Evaluation of ACTN3 R577X and ACE I/D polymorphisms in young Colombian athletes: An exploratory research

MICHELLE ORTIZ¹, ADIS AYALA¹ ✉, JORGE L. PETRO², RODRIGO ARGOTHY³, JONNY GARZÓN³, DIEGO A. BONILLA^{1,4}

¹Francisco José de Caldas District University, Bogotá, Colombia

²University of Córdoba, Montería, Colombia

³Biomechanics Laboratory, Sports Science Center, Coldeportes, Bogotá, Colombia

⁴Research Division, DBSS International SAS, Bogotá, Colombia

ABSTRACT

There are genetic sequences that might be associated with improved athletic performance, such as the α -actinin-3 (ACTN3) R577X polymorphism and the angiotensin-converting enzyme (ACE) insertion/deletion (I/D), which are related to phenotypes of increased muscle strength and endurance, respectively. This STREGA-based cross-sectional study compared the genotype and allele frequencies of the ACTN3 R577X and ACE I/D polymorphisms between Colombian athletes ($n = 37$) and non-athletic controls ($n = 37$). Genotyping was performed using polymerase chain reaction (PCR) and subsequent enzymatic restriction (RFLP). The distribution of the ACTN3 R577X genotype of control and athletic groups met Hardy–Weinberg Equilibrium (HWE) (all $p > .05$); however, in the strength-trained athletes, the distribution of the ACE I/D genotype was not found in HWE. In athletic population, genotype distribution and allele frequencies of the ACTN3 R577X ($n = 37$) was RR: 35.1% ($n = 13$), RX: 54.1% ($n = 20$), XX: 10.8% ($n = 4$), and R: 0.6216 and X: 0.3784, respectively. For ACE I/D ($n = 74$) it was found a genotype distribution of DD: 35.1% ($n = 13$), ID: 24.3% ($n = 9$), II: 40.5% ($n = 15$), and allelic frequencies of D: 62.16% and I: 37.84%. Statistical analysis showed an association between the ACE genotypes with strength, endurance and control groups ($X^2 = 15.3$, $gl = 4$, $p = .004$); however, the ACTN3 R577X polymorphism did not have a significant association ($X^2 = 3.99$, $gl = 4$, $p = .408$). Although studies with a more homogeneous and larger sample size are required, the results of this exploratory study contribute to the genotypic characterization of Colombian athletes with the objective of improving the methodologies and its applications to sports medicine.

Keywords: Physical performance; Genetic variants; Sports medicine; Molecular biology; Sports science.

Cite this article as:

Ortiz, M., Ayala, A., Petro, J.L., Argothy, R., Garzón, J., & Bonilla, D.A. (2022). Evaluation of ACTN3 R577X and ACE I/D polymorphisms in young Colombian athletes: An exploratory research. *Journal of Human Sport and Exercise*, 17(3), 626-639. <https://doi.org/10.14198/jhse.2022.173.14>

✉ **Corresponding author.** Research Group in Biochemistry and Molecular Biology, Chemistry Department, Universidad Distrital Francisco José de Caldas, Carrera 4 #26 D - 54, 110311, Bogotá D.C., Colombia. <https://orcid.org/0000-0003-2830-982X>

E-mail: aayala@udistrital.edu.co

Submitted for publication September 01, 2020.

Accepted for publication November 09, 2020.

Published July 01, 2022 (*in press* November 20, 2020).

JOURNAL OF HUMAN SPORT & EXERCISE ISSN 1988-5202.

© Faculty of Education. University of Alicante.

doi:10.14198/jhse.2022.173.14

INTRODUCTION

Athletes and professionals in sports science seek to constantly improve sports performance in terms of individualization, programming, and periodization of not only exercise but also nutrition and recovery (Kellmann et al., 2018). As molecular knowledge applied to sports sciences advances, the genetic component has become very popular with claims that encompass associations of polymorphisms with sports performance, directing athletes to specialties according to their ability, developing methodologies for early detection of talents, and individualization of training for performance enhancement and injury prevention (Sánchez, Campuzano, Iglesias, & Brugada, 2009). Notwithstanding, it is important to highlight that the science around genetic testing is still considered as an emerging field since the prediction of future sporting performance lacks scientific foundation, it is extremely limited and there is largely non-existent high-quality evidence (Webborn et al., 2015).

Association studies have linked dozens of genetic variants to training responses and sport-related traits, such as strength, skeletal muscle mass, recovery ability and muscle fibre composition (Jones et al., 2016; Pickering, Kiely, Grgic, Lucia, & Del Coso, 2019). The most-studied genes linked to physical performance characteristics of muscle strength and endurance are alpha-actinin-3 (*ACTN3*, Ensembl ID: ENSG00000248746) and angiotensin I converting enzyme (*ACE*, Ensembl ID: ENSG00000159640), respectively, as they are involved in the contractile capacity of skeletal muscle, blood pressure regulation and increased resistance to muscle fatigue (McCauley, Mastana, Hossack, MacDonald, & Folland, 2009). In human, the *ACTN3* protein (UniProtKB: Q08043) is an F-actin cross-linking sarcomeric protein which is thought to anchor actin to a variety of intracellular structures (only in fast-twitch type II muscle fibres) and plays a regulatory role in the coordination of myofibril contraction (MacArthur et al., 2007; North et al., 1999). The R577X polymorphism (rs1815739), which is a genetic variation of an arginine (R) for a premature stop codon (X) in the *ACTN3* gene, has been strongly associated with high muscle contraction capacity in power and strength sports (Gonzalez et al., 2013; N. Yang et al., 2003) but less in endurance activities (Saunders et al., 2007). On the other hand, the rs1799752 polymorphism in the *ACE* gene is characterized by a sequence variation, which exerts distinct influence on tissue expression and serum activity of the *ACE* protein (UniProtKB: P12821). This protein has an important role in regulating blood volume, blood pressure, and electrolyte balance, besides being necessary to sustain physiological demands when muscle metabolism is increased during exercise (McCauley, Mastana, & Folland, 2010). This polymorphism is based on the presence (insertion [allele I]) or absence (deletion [allele D]) of a nonsense DNA fragment (Alu sequence of 287 bp) in intron 16 (Rigat et al., 1990), represented hereinafter as I/D.

Background

In Colombia, research in this field is scarce even though in the last twenty years many genetic markers related to sports performance have been identified; however, the *ACTN3* and *ACE* genotypes are the most commonly tested by genetic companies and research groups. In high-altitude Colombian population (above 2,500 meters), Enciso-Castellanos (Enciso-Castellanos, 2006) observed no significant difference between 29 sedentary subjects and 27 high-performance endurance athletes when evaluating the genotypes of lipoprotein lipase (LPL), peroxisome proliferator-activated receptor alpha (PPAR- α), β -3 adrenergic receptor, and *ACE* I/D; however, slight trends in allelic frequencies between the groups were evidenced. Neither was found a direct relationship of any of the genetic markers that showed that the VO_{2peak} depended on these polymorphisms. Valderrama-Aguirre and Endo (Valderrama, 2009) reported, by the first time, information related to the allelic frequency and genotype distribution of *ACE* I/D in young adults from Cali, Valle del Cauca (Colombia). The 60-participants group followed the Hardy-Weinberg equilibrium (HWE: $X^2 = 0.60$ and $p = .805$) and the observed allelic frequency was 50% (heterozygosity = 52%). These results were of great

importance on establishing lines of translational research in Colombia. In this sense, the same research group (Vallejo, 2009) performed the genotyping of 63 young individuals (62% women and 38% men) from general population and found a prevalence of 59% for the *ACTN3* R577X polymorphism (heterozygosity = 57%). This allowed the authors to conclude that the mutation was highly prevalent in the studied sample; actually, besides being one of the largest reported to date in Colombia, this study was the by the first time the prevalence of the R577X mutation in a South American country. Finally, in a recent explorative study in 26 high-performance Colombian weightlifters, Stucky-Byler and Rodríguez-Buitrago (Stucky-Byler, 2018) compared genotype outcomes for *ACTN3* in terms of performance and showed a significant relationship between the RX genotype and sporting achievement, the Sinclair coefficient and an empiric comparison with the lifting world record. Notwithstanding, the results were not consistent with those from previous studies, where the RR genotype was associated with better sports performance (Jacob, Spiteri, Hart, & Anderton, 2018). These results raise the need to explore the hypothesis that the RR genotype is more related to performance in speed and power sports, while the RX genotype is more related to strength and power sports, especially in sports where body weight is a key factor in competition (Vallejo, 2009).

Objective

Thus, the aim of this research was to carry out an exploratory study of the genotype distribution and allelic frequency of the *ACTN3* R577X and *ACE* I/D polymorphisms in performance athletes, belonging to the Bogotá athletics league, in order to contribute to Colombian molecular characterization and engage new research projects related to sports performance.

MATERIALS AND METHODS

Study design

A cross-sectional study was performed to explore the genotype distribution and allelic frequency of the *ACTN3* R577X and *ACE* I/D polymorphisms in young Colombian athletes. The guidelines for Strengthening the Reporting of Genetic Association studies (STREGA) (Little et al., 2009), an extension of the STROBE statement, were followed for reporting the results of this study.

Setting

This study was carried out between July 2018 and November 2019 within the framework of an undergraduate project. Due to the sports calendar of the COLDEPORTES leagues and irregular availability of the athletes, several visits to collect the blood samples by groups of five athletes were necessary. The study protocol was approved by the Research and Ethical Committee of the Universidad Distrital Francisco José de Caldas (code: CIDC-0334-2017) in accordance with the latest version of the Declaration of Helsinki (Association, 2013). All subjects provided written informed consent before participation. Information regarding the purpose of the study, potential risks, and protection of the subjects' rights were provided to all participants.

Subjects

Eighty-nine subjects (among athletes and control subjects) volunteered and were eligible to participate in this study. The athletic population was either recruited at the leagues' facilities or by coaches that knew about previous participation in other investigations. The strength and endurance athletes belonged either to the *Liga de Atletismo de Bogotá* or to the *Escuela de Cadetes de Policía Nacional General Francisco de Paula Santander*. Inclusion criteria for athletes' selection were as follows: i) age 18–30 years; ii) no previous evidence of muscle, cardiac or kidney disease; iii) active sports competitors at the national professional sports leagues recognized by COLDEPORTES and the Colombian Olympic Committee; and iv) with more than two years of strength or endurance sports experience. On the other hand, apparently healthy college students

from several Colombian universities (mainly Universidad Distrital Francisco José de Caldas) were recruited via an announcement posted in several social networks. Exclusion criteria for all subjects were: smoking, taking medications or having had a musculoskeletal injury in the six months prior to the study.

Variables

Genetic variants were considered as main outcomes (nonsense single nucleotide polymorphism for the ACTN3 gene and I/D of an Alu-type sequence in the ACE gene). The following variables were also measured and/or reported: body mass (kg), stature (cm), sport discipline, age and sex.

Data sources / Measurement

All blood samples from the athletic population were collected at the *Centro de Ciencias del Deporte* in the *Centro de Alto Rendimiento COLDEPORTES* facilities during a 25-min assessment session per group of five subjects before routinely physical evaluation. Healthy control subjects visited the Biochemistry and Molecular Biology laboratory at Universidad Distrital Francisco José de Caldas for a 20-min assessment session, where the anthropometric evaluation was performed and the blood samples were taken.

Anthropometry

Body mass was measured without shoes to the nearest 0.05 kg using a digital scale (Seca 703, Hamburg, Germany). An adult portable stadiometer (Seca 213, Hamburg, Germany) was used to measure the stature according to the ISAK protocol (Esparza-Ros, Vaquero-Cristóbal, & Marfell-Jones, 2019).

DNA extraction and quantification

Ten millilitres (10 mL) of a blood sample from the forearm veins were collected by certified laboratory technicians from *COLDEPORTES* and *DBSS International* in 15-mL tubes containing an ethylenediaminetetraacetic acid (EDTA, 10 mM) anticoagulant and were stored at 4°C. Genomic DNA for genetic analysis was isolated from peripheral blood using a salting-out method modified by Ayala (Ayala, 1997). Subsequently, DNA concentration (mg/ml) was quantified at 260 nm in a spectrophotometer (Hitachi Genetic Systems, Gene Spec I). The purity of DNA was determined by measuring the ratio of 260/280 nm and the range of 1.6 - 2 were considered as pure (Eschbach, Hofmann, Maerz, Maier, & Sitte, 1990). The average DNA concentration after the extraction from the 74 samples was $0.168 \pm 0.12 \mu\text{g}/\mu\text{L}$ with a purity percentage of $69.0 \pm 16.24 \%$. DNA samples were genotyped using the polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) method.

ACTN3 R577X (rs1815739) Genotyping

The standardized amplification conditions for the *ACTN3* gene were: magnesium-free Taq buffer 1.6X, 1.6 mM MgCl_2 , 210 μM dNTPs, 0.5 μM of forward primer (5'CTGTTGCCTGTGGTAAGTGGG'3), 0.5 μM of reverse primer (5'TGGTCACAGTATGCAGGAGGG'3), 1.25 U of Taq DNA polymerase and 200 ng of genomic DNA, for a final total volume of 40 μL . PCR conditions for amplification were the following: i) initial denaturation at 94°C (5 min); ii) 30 cycles of denaturation at 94°C (1 min), annealing at 56°C (1.5 min) and extension at 72°C (1 min); and iii) final extension at 72°C (4 min) (MiniAmp™ Plus Thermal Cycler, Singapore). The amplification products of each sample were digested by the restriction enzyme DdeI (5'-C▼TNA▼G-3') for 90 min in a 30 μL reaction mix at 37°C in duplicate (1.5 U DdeI, 1X buffer D (Promega, USA), 0.1 g/ μL BSA, and 1 μg of amplified DNA). The negative control was processed with $\text{H}_2\text{O}_{\text{dE}}$ and Bacteriophage Lambda DNA was used as the positive control. The restrictions fragments were separated by horizontal electrophoresis in a 2.4% agarose gel (NuSieve™, Lonza) stained with ethidium bromide (0.5 $\mu\text{g}/\text{ml}$) at 1X TBE and run at 100 V for 120 min. Afterward, gels were placed on a UV transilluminator and recorded photographically.

From the amplification conditions, a 291 bp DNA segment from the exon 16 of the *ACTN3* gene was obtained. The amplicon size corresponded to the previous results of Mills et al. (Mills et al., 2001). After enzymatic restriction of the amplicon, control subjects and athletes that carry the R allele presented specific molecular products of 205 and 86 bp, whilst the X allele showed three fragment products that were detected at 108, 97 and 86 bp. Thus, the RR homozygous genotype produced two bands of 205 and 86 bp, while heterozygous individuals RX showed fragments of 205, 108, 97 and 86 bp. The mutated homozygotes XX presented fragments of 108, 97 and 86 bp. The sizes obtained in this study were in agreement with those reported by Eynon et al. (N. Eynon et al., 2009).

ACE I/D (rs1799752) Genotyping

The reaction mixture for amplification of the *ACE* gene in intron 16 was: magnesium-free Taq buffer 1.6X, 1.7 mM of MgCl₂, 225 µM dNTPs, 0.7 µM of forward primer (5'CTGGAGACCACTCCCATCCTTTCT'3), 0.69 µM of reverse primer (5'ATGTGGCCATCACATTCGTCAGAT'3), and 1.25 U of Taq DNA polymerase and 200 ng of genomic DNA, for a final total volume of 40 µL. The PCR conditions for amplification were the following: i) initial denaturation at 94°C (5 min); ii) 30 cycles of denaturation at 94°C (1 min), annealing at 64°C (1.5 min) and extension at 72°C (1 min); and iii) final extension at 72°C (4 min) (MiniAmp™ Plus Thermal Cycler, Singapore). The amplification products of each sample were separated by electrophoresis in a 2.4% agarose gel (NuSieve™, Lonza) stained with ethidium bromide (0.5 µg/ml) at 1X TBE and run at 90 V for 100 min. Afterward, gels were placed on a UV transilluminator and recorded photographically.

The *ACE* I/D was identified as the absence (deletion or D allele) or presence (insertion and I allele) of 287 base pairs in intron 16 of the gene (Ribas et al., 2017). This was confirmed by the presence of a 490 bp segment for alleles II, whilst a 190 pb fragment was detected for alleles DD.

Study size

A non-probability convenience sampling from COLDEPORTES-recognized leagues (strength and endurance athletes) and Colombian universities with branch in Bogotá (non-athletes) was used, considering the difficulty in procuring a large sample of athletes and the 1:1 allocation ratio design.

Statistical analysis

The descriptive statistics are expressed as mean (\bar{X}) and standard deviation (SD). The HWE and the association between the genotype (*ACTN3* and *ACE*) and the main groups (strength, endurance and control) were carried out by means of the chi-square test (X^2). The significance level assumed for all the tests was $p < .05$. The statistical procedures were performed with SPSS Statistics 25 for Windows (IBM Corp., Armonk, NY, USA).

RESULTS

Participants

After the evaluation of inclusion criteria, eighty-five subjects (45 athletes and 40 control subjects) were considered for data collection; however, due to the competition schedule four previously confirmed athletes did not assist to the assessment session. Three students were not measured because of education duties. Therefore, in this study we evaluated the genotype distribution and allelic frequency of seventy-four subjects; 37 athletes (27 strength- and 10 endurance-trained) and 37 apparently healthy untrained subjects. Figure 1 shows the selection, grouping and final data analysis of the individuals in a flow diagram, and the distribution of the sample by sex and groups is shown in Table 1.

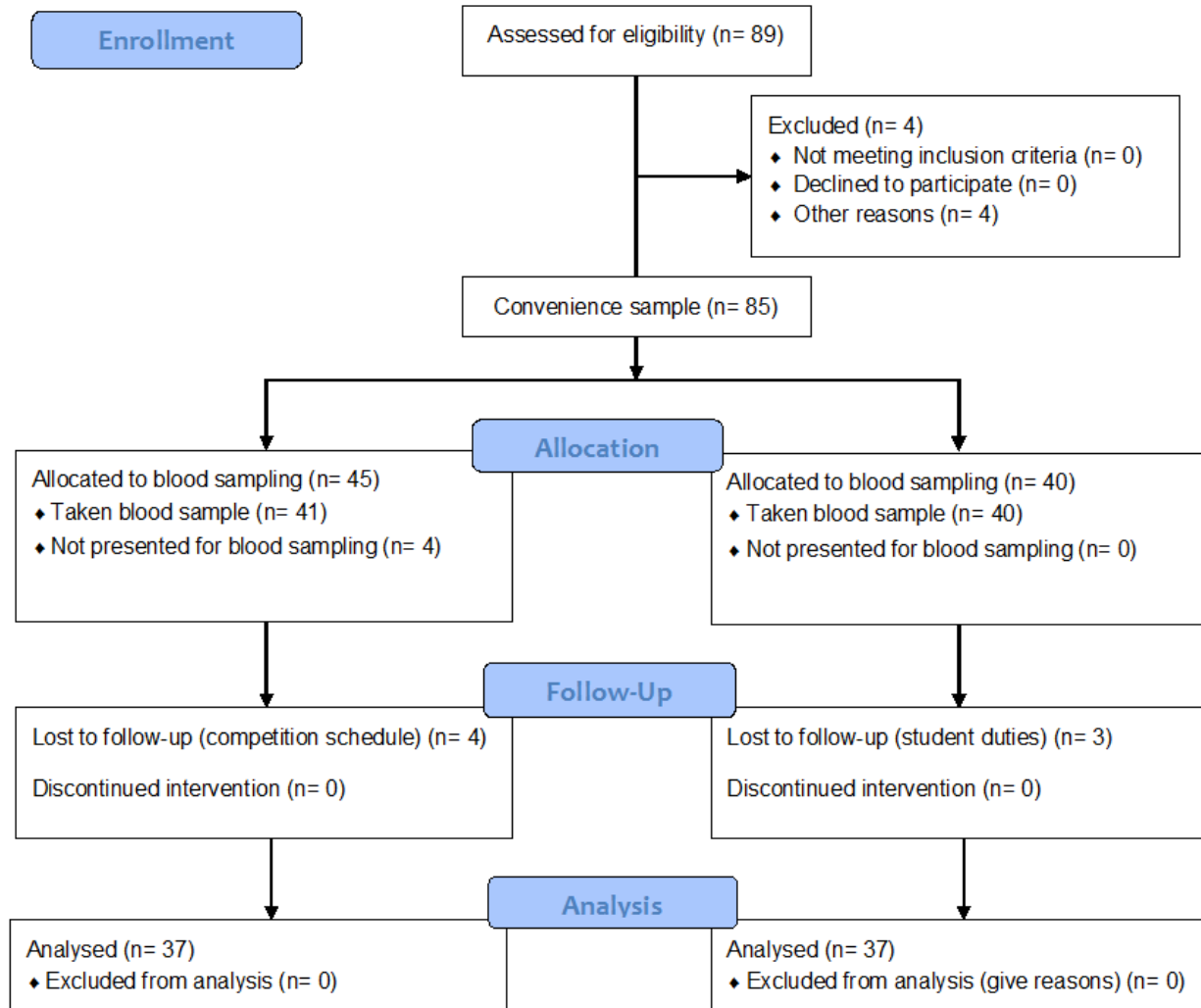


Figure 1. Flowchart of participant selection for the analysis.

Table 1. Subjects participating in the study by sex and groups.

Group	Sex		Total
	F	M	
Athletes	10 (27.0)	27 (73.0)	37 (100)
Strength	7 (25.9)	20 (74.1)	27 (100.0)
Endurance	3 (30.0)	7 (70.0)	10 (100.0)
Control	15 (40.5)	22 (59.5)	37 (100.0)
Total	25 (33.8)	49 (66.2)	74 (100.0)

Data expressed as frequency (% within the group). F, female; M, male.

Descriptive data

The strength athletes were involved in speed (22), jump (2) and javelin throw (3) disciplines, while the endurance athletes were long-distance runners (10). Data for anthropometric measurements of the participants in this investigation are outlined in Table 2.

Table 2. Anthropometric and physical characteristics.

Variable (units)	Athletes (n = 37)		Controls (n = 37)	p-value
	Strength (n = 27)	Endurance (n = 10)		
Age (years)	21.3 ± 2.0	22.9 ± 3.3	21.9 ± 2.6	.325
Body mass (kg)	66.6 ± 9.7	60.0 ± 8.7	64.6 ± 11.0	.328
Height (cm)	173.2 ± 8.3	170.5 ± 10.4	168.1 ± 8.2	.109
BMI (kg·m ⁻²)	22.09 ± 1.698	20.55 ± 1.8*	22.75 ± 2.5*	.022

Data are presented as mean ± SD. Body mass index; p-value from Kruskal-Wallis; * difference found between Control and Endurance group ($p < .013$).

Outcome data

ACTN3 R577X (rs1815739) Genotype and Allele Frequencies

ACTN3 genotype distribution in the strength-athletes showed a higher frequency of the R allele (FA; R/X = 0.65/0.35) and a predominant RX and RR genotype (55.6% and 37%, respectively). On the other hand, the endurance-trained athletes had a lower frequency of the R allele (FA; R/X 0.55/0.45) with a homozygote genotype frequency of 30%. Control subjects presented a close allelic frequency (FA; R/X 0.61/0.39) in relation to the sport disciplines but showed a higher RR genotype frequency in comparison to strength- and endurance-trained athletes. In addition, control group had a decrease in heterozygote genotype frequency RX with respect to athletes (Table 3). The ACTN3 R577X genotype frequencies were in HWE in all groups ($p > .05$).

Table 3. ACTN3 R577X genotype distribution and allele frequencies.

Genotype Distribution	All athletes(n = 37)	Strength (n = 27)	Endurance (n = 10)	Control (n = 37)
RR	13 (35.1 %)	10 (37%)	3 (30%)	16 (43.24%)
RX	20 (54.1%)	15 (55.6%)	5 (50%)	13 (35.14%)
XX	4 (10.8%)	2 (7.4%)	2 (20%)	8 (21.62%)
HWE p-value	0.365	0.257	0.975	0.11
Allelic Frequency				
R	46 (62.16%)	35 (64.8%)	11 (55%)	45 (60.8%)
X	28 (37.84%)	19 (35.2%)	9 (45%)	29 (39.2%)

HWE, Hardy-Weinberg equilibrium; distribution of the genotype expressed as frequency (percentage).

ACE I/D (rs1799752) Genotype and Allele Frequencies

Table 4. ACE I/D genotype distribution and allele frequencies.

Genotype Distribution	All athletes(n = 37)	Strength (n = 27)	Endurance (n = 10)	Control (n = 37)
DD	13 (35.1 %)	11 (40.7%)	2 (20%)	10 (27%)
DI	9 (24.3%)	3 (11.1%)	6 (60%)	20 (54.1%)
II	15 (40.5%)	13 (48.2%)	2 (20%)	7 (18.9%)
HWE p-value	0.002	<0.005	0.739	0.591
Allelic Frequency				
D	35 (62.16%)	25 (46.3%)	10 (50%)	40 (54.1%)
I	39(37.84%)	29 (53.7%)	10 (50%)	34 (45.9%)

HWE, Hardy-Weinberg equilibrium; distribution of the genotype expressed as frequency (percentage).

ACE DD genotype distribution in strength-athletes was approximately 2- and 1.5-fold in comparison to endurance-athletes and control subjects. The homozygote II genotype distributions for strength-, endurance-

trained and control individuals were 48.2%, 20%, and 18.9%, respectively (Table 4). ACTN3 R577X and ACE I/D genotype frequencies were in HWE in the endurance and control group ($p = .739$ and $.591$, respectively), but not in the strength group ($p < .05$).

DISCUSSION

In this exploratory study, we evaluated the genotype distribution and allelic frequency of the ACTN3 R577X and ACE I/D polymorphisms in a group of Colombian strength- and endurance-athletes. We also performed the genotyping of a matched-size sample of healthy non-athletic individuals. The results showed that all group met the HWE for ACTN3 R577X genotype (all $p > .05$), but this was not found for the ACE I/D genotype in the athlete group (.002), particularly for the strength group ($p < .05$). In the strength-trained athletes, it was observed a higher predominance of the RX genotype (55.6%), in comparison with the RR (37%) and XX (7.4%) genotypes. These athletes also had a higher distribution of the R than the X allele (64.8% and 35.2%, respectively). Interestingly, the endurance athletes had a similar genotype distribution for RR and RX (see Table 3) but important differences were obtained on both the XX genotype (20% endurance vs. 7.4% in the strength-group) and the allelic frequency (R = 55%, X = 45%). This behaviour of the data is in accordance with previous reports in athletes trained for strength, power or high-intensity short-rest efforts (Nir Eynon et al., 2013; Lammi, Tharabenjasin, Pabalan, & Jarjanazi, 2019; Ribas et al., 2017). For example, Eynon et al. (Nir Eynon et al., 2013) showed a genotype distribution on elite and national-level strength/power-athletes from Spain ($n = 119$, RR: 31%, RX: 55%, and XX: 13%), Poland ($n = 178$, RR: 40%, RX: 52%, and XX: 8%) and Russia ($n = 82$, RR: 40%, RX: 48%, and XX: 12%) that were similar to our results. Ribas et al. (Ribas et al., 2017) also reported analogous findings in a Brazilian population of elite fighters; notwithstanding, it is interesting to note that our results coincide only with the genotype distribution of the percussion group (athletes who practice Karate, Taekwondo, Muay Thai, boxing – $n = 14$, RR: 37.7%, RX: 57%, and XX: 7.2%) and not with the grappling group (judo, Brazilian Jiu-Jitsu, Greco-Roman wrestling – $n = 23$, RR: 52.2%, RX: 26.1%, and XX: 21.7%). This might be due to the high-intensity short-term movements with greater strength and power, which are features of the percussion-based combat sports. In fact, the ACTN3 R577X polymorphism has been postulated as a potential marker for power performance (Nir Eynon et al., 2013) and there is an association between the number of type II muscle fibres and the RR and RX genotype (Vincent et al., 2007). Hence, the higher presence of the RX (followed by RR) genotype reported in our study for the strength-athletes might be related to a higher expression of ACTN3 protein, which is particularly involved in the optimization of muscle contraction in fast-twitch fibres. Furthermore, the R allele has been associated with a conservation of muscle mass (Galeandro et al., 2017) and a recent meta-analysis has clearly presented the associations between the RX and RR genotype / R allele with power performance (Lammi et al., 2019). This is in agreement with the results of Stucky-Byler and Rodríguez-Buitrago (Stucky-Byler, 2018), who demonstrated a relationship between the RX genotype and the strength/power performance in Colombian weightlifters.

On the other hand, endurance sports are characterized by a relatively low-moderate intensity and long-duration events, where oxidative metabolism is the predominant source for ATP resynthesis during exercise (Petro & Bonilla, 2015). The insertion of the 287 base-pair Alu sequence in the ACE gene has been associated to slow-twitch type I muscle fibres (Papadimitriou et al., 2016), and is reported to account for the $\approx 47\%$ of fluctuations in the protein activity in plasma (Sabir et al., 2019; Woods et al., 2001). The insertion of this sequence has been related to less vasoconstriction due to a decrease in the ACE serum levels and activity and, therefore, higher oxygen availability and nutrients delivery for muscle fibres in contraction (Gunel et al., 2014). In Tunisian athletes the I and D alleles of the ACE polymorphism have been associated with high level of human endurance and power performance, respectively (Znazen et al., 2015). In this sense,

Gonzalez et al. (Gonzalez et al., 2013) published a meta-analysis which consistently provided more solid evidence for associations between *ACE* II genotype and endurance events. In our group of endurance-trained athletes, it was observed a higher predominance of the DI genotype (60%), in comparison to the DD (20%) and II (20%) genotypes. These athletes also had an equal distribution of both the D and I allele (50% each one). Notwithstanding, the very low number of these athletes ($n = 10$) makes difficult to have an objective interpretation of the results; thus, the high frequency observed might account for the variability in the athletes' population, which enforces the recommendation of focusing genetic studies on a large cohort within a single sport instead of combining several sports with varied demands and athletes' characteristics (Heffernan et al., 2016). In fact, in team sports, the players' position has shown to be associated to a different genotype distribution (Bell et al., 2010).

Our study also found a combination of *ACTN3* and *ACE* gene variants for RR and DD genotypes in four athletes, which have been linked to competitive advantage, as Galeandro et al. (Galeandro et al., 2017) demonstrated in a group of football players with clear co-occurrence of the *ACTN3* RR and *ACE* DD genotypes. Similarly, four athletes showed *ACTN3* XX and *ACE* II genotypes, which might be related to resisting muscle fatigue, which is in agreement with previous data (Nir Eynon et al., 2013). In this sense, using genetic models, Papadimitriou et al. (Papadimitriou et al., 2016) established that the *ACTN3* R allele and *ACE* D allele dominant model account for 0.92 % and 1.48 % of sprint time variance, respectively, in a large, performance-homogenous cohort of elite Australian, Brazilian, Greek, Jamaican, Italian, Polish, Russian, Lithuanian, Spanish and US sprinters. Although some research have established that the genetic background of *ACTN3* R577X and/or *ACE* I/D polymorphisms play an important role in sporting potential, which might explain why some individuals may be better adapted to specific physical training or performance (Chiu et al., 2019; Coelho et al., 2018; Del Coso et al., 2019; Kikuchi et al., 2015; Li et al., 2017; Massidda et al., 2019; R. Yang et al., 2017; Znazen et al., 2015), readers should note that other studies have reported no association between the *ACTN3* R577X and/or *ACE* I/D polymorphism and athletic performance (Falahati & Arazi, 2019; Koku et al., 2019; Maciejewska-Skrendo, Cięższyk, Chycki, Sawczuk, & Smółka, 2019; Massidda et al., 2015; Miyamoto, Miyamoto-Mikami, Hirata, Kimura, & Fuku, 2018; Moreno-Pérez, Machar, Sanz-Rivas, & Del Coso, 2020; Papadimitriou et al., 2018). For example, the findings of Orysiak et al. (Orysiak et al., 2018) do not support an influential role of *ACE* I/D and *ACTN3* R577X genotypes (alone or in combination) on power/strength performance in elite Polish athletes. In this regard, these two polymorphisms together, separately, or part of an algorithm do not predict training response due to the polygenic nature of the adaptation to exercise; therefore, we advise that personalized training derived from genetic testing have to be based on clear, confident, robust, and reproducible studies, specially while genome-wide association studies are developed to confirm the type of relation between genotypes and physical / performance outcomes. Actually, the current general consensus among sport and exercise genetics researchers is that is too early to recommend direct-to-consumer genetic testing in order to program training or for talent identification or selecting children or adolescents (Webborn et al., 2015).

Limitations

This study has several limitations that should be highlighted. As an individual study, it may be hampered by sample size. Also, the variability in the athletes' population makes necessary more studies with a larger and more homogeneous cohort that allow coming with strong conclusions. Additionally, due to technical issues we were not able to report associations to morphological and performance outcomes. Finally, given the lack in replicated scientific evidence for the studied polymorphisms, besides of controversial results, exercise and athletic population can learn about their genetic distribution but the consensus is that the predictive value for training or talent identification is low (Webborn et al., 2015). Notwithstanding, our study add information to

the scientific literature about the genotype distribution and allelic frequency in Colombian athletes, which might encourage future molecular studies in the field.

CONCLUSIONS

Physical performance is conditioned by factors such as nutrition, exercise, environment, and biological elements that are related to the inheritance of individual characters. Despite the small sample, our results of genotype distribution and allelic frequency for *ACTN3* R577X and *ACE* I/D polymorphisms in strength-trained athletes are in accordance with previous reports for high-intensity intermittent exercisers and power/strength athletes. These athletes had a higher distribution of the R than the X allele in the *ACTN3* gene. The very low number of endurance-trained athletes makes difficult to have an objective interpretation of our results. This exploratory study contributes to the molecular characterization of Colombian athletes, with an unprecedented report of the genotypic distribution and allelic frequency of *ACTN3* R577X and *ACE* I/D. More research is needed with the aim of improving the methodologies and its applications to sports medicine.

AUTHOR CONTRIBUTIONS

AA served as the lab coordinator and project manager. MO, AA and DAB conceived and designed the experiments. MO, AA, DAB, RA, JG and SA assisted in data collection. MO, AA and JLP analysed the data. MO, AA and DAB wrote the paper. DAB and JLP assisted in the statistics advice, discussion analysis, and manuscript preparation and review for submission. All authors read and approved the final manuscript.

SUPPORTING AGENCIES

This study was supported by the Centro de Investigaciones y Desarrollo Científico at Universidad Distrital Francisco José de Caldas. The authors would like to thank all athletes that participated in the study, the professionals at COLDEPORTES (Sandra Moreno), and all DBSS International fellows that collaborated.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

REFERENCES

- Ayala, A. (1997). Estudio del gen que codifica la enzima N-Acetil-Galactosamina-6-Sulfato-Sulfatasa en personas normales y pacientes colombianos con la enfermedad de Morquio. (Unpublished master dissertation), Universidad de los Andes, Bogotá, Colombia.
- Bell, W., Colley, J., Gwynne, J., Glazier, P., Evans, W. D., & Darlington, S. E. (2010). ACE ID genotype and leg power in rugby union players. *Journal of sports medicine and physical fitness*, 50(3), 350.
- Chiu, Y.-H., Lai, J.-I., Tseng, C.-Y., Wang, S.-H., Li, L.-H., Kao, W.-F., . . . Hsieh, C.-Y. (2019). Impact of angiotension I converting enzyme gene I/D polymorphism on running performance, lipid, and biochemical parameters in ultra-marathoners. *Medicine*, 98(29), e16476. <https://doi.org/10.1097/MD.00000000000016476>
- Coelho, D., Pimenta, E., Rosse, I., de Castro, B., Becker, L., de Oliveira, E., . . . Garcia, E. (2018). Evidence for a Role of ACTN3 R577X Polymorphism in Football Player's Career Progression. *International Journal of Sports Medicine*, 39(14), 1088-1093. <https://doi.org/10.1055/a-0753-4973>

- Del Coso, J., Moreno, V., Gutiérrez-Hellín, J., Baltazar-Martins, G., Ruíz-Moreno, C., Aguilar-Navarro, M., . . . Lucía, A. (2019). ACTN3 R577X Genotype and Exercise Phenotypes in Recreational Marathon Runners. *Genes*, 10(6), 413. <https://doi.org/10.3390/genes10060413>
- Enciso-Castellanos, S. (2006). LDL y receptor β -3 adrenérgico y su correlación con el VO₂ pico en individuos sedentarios y entrenados residentes en altura intermedia. (Unpublished master dissertation), Universidad Nacional de Colombia, Bogotá, Colombia.
- Eschbach, S., Hofmann, C., Maerz, M., Maier, U. G., & Sitte, P. (1990). *Molecular Cloning. A Laboratory Manual*. 2. Auflage. Hrsg. von J. Sambrook, EF Fritsch, T. Maniatis, Cold Spring Harbor Laboratory Press, Cold Spring Harbour 1989, \$115. ISBN 0-87969-309-6. *Biologie in unserer Zeit*, 20(6), 285-285. <https://doi.org/10.1002/biuz.19900200607>
- Esparza-Ros, F., Vaquero-Cristóbal, R., & Marfell-Jones, M. (2019). International Standards for Anthropometric Assessment. In Murcia, Spain: The International Society for the Advancement of Kinanthropometry.
- Eynon, N., Duarte, J. A., Oliveira, J., Sagiv, M., Yamin, C., Meckel, Y., . . . Goldhammer, E. (2009). ACTN3R577X Polymorphism and Israeli Top-level Athletes. *International Journal of Sports Medicine*, 30(09), 695-698. <https://doi.org/10.1055/s-0029-1220731>
- Eynon, N., Hanson, E. D., Lucia, A., Houweling, P. J., Garton, F., North, K. N., & Bishop, D. J. (2013). Genes for Elite Power and Sprint Performance: ACTN3 Leads the Way. *Sports Medicine*, 43(9), 803-817. <https://doi.org/10.1007/s40279-013-0059-4>
- Falahati, A., & Arazi, H. (2019). Association of ACE gene polymorphism with cardiovascular determinants of trained and untrained Iranian men. *Genes and Environment*, 41(1). <https://doi.org/10.1186/s41021-019-0126-7>
- Galeandro, V., Notamicola, A., Bianco, A., Tafuri, S., Russo, L., Pesce, V., . . . Petruzzella, V. (2017). ACTN3/ACE genotypes and mitochondrial genome in professional soccer players performance. *Journal of Biological Regulators and Homeostatic Agents*, 31(1), 207-213.
- Gonzalez, G. E., Ma, F., Yang, Y., Li, X., Zhou, F., Gao, C., . . . Gao, L. (2013). The Association of Sport Performance with ACE and ACTN3 Genetic Polymorphisms: A Systematic Review and Meta-Analysis. *PLoS One*, 8(1), e54685. <https://doi.org/10.1371/journal.pone.0054685>
- Gunel, T., Gumusoglu, E. C. E., Hosseini, M. K., Yilmazyildirim, E. D. A., Dolekcap, I., & Aydinli, K. (2014). Effect of angiotensin I-converting enzyme and α -actinin-3 gene polymorphisms on sport performance. *Molecular Medicine Reports*, 9(4), 1422-1426. <https://doi.org/10.3892/mmr.2014.1974>
- Heffernan, S. M., Kilduff, L. P., Erskine, R. M., Day, S. H., McPhee, J. S., McMahan, G. E., . . . Williams, A. G. (2016). Association of ACTN3 R577X but not ACE I/D gene variants with elite rugby union player status and playing position. *Physiological Genomics*, 48(3), 196-201. <https://doi.org/10.1152/physiolgenomics.00107.2015>
- Jacob, Y., Spiteri, T., Hart, N., & Anderton, R. (2018). The Potential Role of Genetic Markers in Talent Identification and Athlete Assessment in Elite Sport. *Sports (Basel)*, 6(3), 88. <https://doi.org/10.3390/sports6030088>
- Jones, N., Kiely, J., Suraci, B., Collins, D., de Lorenzo, D., Pickering, C., & Grimaldi, K. (2016). A genetic-based algorithm for personalized resistance-training. *Biology of Sport*, 33(2), 117-126.
- Kellmann, M., Bertollo, M., Bosquet, L., Brink, M., Coutts, A. J., Duffield, R., . . . Beckmann, J. (2018). Recovery and Performance in Sport: Consensus Statement. *International Journal of Sports Physiology and Performance*, 13(2), 240-245. <https://doi.org/10.1123/ijsp.2017-0759>
- Kikuchi, N., Miyamoto-Mikami, E., Murakami, H., Nakamura, T., Min, S.-K., Mizuno, M., . . . Fuku, N. (2015). ACTN3R577X genotype and athletic performance in a large cohort of Japanese athletes. *Eur J Sport Sci*, 16(6), 694-701. <https://doi.org/10.1080/17461391.2015.1071879>

- Koku, F. E., Karamızrak, S. O., Çiftçi, A. S., Taşlıdere, H., Durmaz, B., & Çoğulu, Ö. (2019). The relationship between ACTN3 R577X gene polymorphism and physical performance in amateur soccer players and sedentary individuals. *Biology of Sport*, 36(1), 9-16. <https://doi.org/10.5114/biolsport.2018.78900>
- Lammi, M. J., Tharabenjasin, P., Pabalan, N., & Jarjanazi, H. (2019). Association of the ACTN3 R577X (rs1815739) polymorphism with elite power sports: A meta-analysis. *PLoS One*, 14(5), e0217390. <https://doi.org/10.1371/journal.pone.0217390>
- Li, Y., Wang, L. Q., Yi, L., Liu, J., Hu, Y., Lu, Y., & Wang, M. (2017). ACTN3 R577X genotype and performance of elite middle-long distance swimmers in China. *Biology of Sport*, 1, 39-43. <https://doi.org/10.5114/biolsport.2017.63731>
- Little, J., Higgins, J. P., Ioannidis, J. P., Moher, D., Gagnon, F., von Elm, E., . . . Birkett, N. (2009). Strengthening the reporting of genetic association studies (STREGA): an extension of the strengthening the reporting of observational studies in epidemiology (STROBE) statement. *J Clin Epidemiol*, 62(6), 597-608 e594. <https://doi.org/10.1016/j.jclinepi.2008.12.004>
- MacArthur, D. G., Seto, J. T., Raftery, J. M., Quinlan, K. G., Huttley, G. A., Hook, J. W., . . . North, K. N. (2007). Loss of ACTN3 gene function alters mouse muscle metabolism and shows evidence of positive selection in humans. *Nature Genetics*, 39(10), 1261-1265. <https://doi.org/10.1038/ng2122>
- Maciejewska-Skrendo, A., Ciężczyk, P., Chycki, J., Sawczuk, M., & Smółka, W. (2019). Genetic Markers Associated with Power Athlete Status. *J Hum Kinet*, 68(1), 17-36.
- Massidda, M., Bachis, V., Corrias, L., Piras, F., Scorcu, M., Culigioni, C., . . . Calò, C. M. (2015). ACTN3 R577X polymorphism is not associated with team sport athletic status in Italians. *Sports Medicine - Open*, 1(1). <https://doi.org/10.1186/s40798-015-0008-x>
- Massidda, M., Voisin, S., Culigioni, C., Piras, F., Cugia, P., Yan, X., . . . Calò, C. M. (2019). ACTN3 R577X Polymorphism Is Associated With the Incidence and Severity of Injuries in Professional Football Players. *Clinical Journal of Sport Medicine*, 29(1), 57-61. <https://doi.org/10.1097/JSM.0000000000000487>
- McCauley, T., Mastana, S. S., & Folland, J. P. (2010). ACE I/D and ACTN3 R/X polymorphisms and muscle function and muscularity of older Caucasian men. *European Journal of Applied Physiology*, 109(2), 269-277. <https://doi.org/10.1007/s00421-009-1340-y>
- McCauley, T., Mastana, S. S., Hossack, J., MacDonald, M., & Folland, J. P. (2009). Human angiotensin-converting enzyme I/D and α -actinin 3 R577X genotypes and muscle functional and contractile properties. *Experimental Physiology*, 94(1), 81-89. <https://doi.org/10.1113/expphysiol.2008.043075>
- Mills, M., Yang, N., Weinberger, R., Vander Woude, D. L., Beggs, A. H., Eastal, S., & North, K. (2001). Differential expression of the actin-binding proteins, α -actinin-2 and -3, in different species: implications for the evolution of functional redundancy. *Human Molecular Genetics*, 10(13), 1335-1346. <https://doi.org/10.1093/hmg/10.13.1335>
- Miyamoto, N., Miyamoto-Mikami, E., Hirata, K., Kimura, N., & Fuku, N. (2018). Association analysis of the ACTN3 R577X polymorphism with passive muscle stiffness and muscle strain injury. *Scand J Med Sci Sports*, 28(3), 1209-1214. <https://doi.org/10.1111/sms.12994>
- Moreno-Pérez, V., Machar, R., Sanz-Rivas, D., & Del Coso, J. (2020). ACTN3 R577X Genotype in Professional and Amateur Tennis Players. *Journal of Strength and Conditioning Research*, 34(4), 952-956. <https://doi.org/10.1519/JSC.00000000000003501>
- North, K. N., Yang, N., Wattanasirichaigoon, D., Mills, M., Eastal, S., & Beggs, A. H. (1999). A common nonsense mutation results in α -actinin-3 deficiency in the general population. *Nature Genetics*, 21(4), 353-354. <https://doi.org/10.1038/7675>
- Orysiak, J., Mazur-Różycka, J., Busko, K., Gajewski, J., Szczepanska, B., & Malczewska-Lenczowska, J. (2018). Individual and Combined Influence of ACE and ACTN3 Genes on Muscle Phenotypes in

- Polish Athletes. *Journal of Strength and Conditioning Research*, 32(10), 2776-2782. <https://doi.org/10.1519/JSC.0000000000001839>
- Papadimitriou, I. D., Lockett, S. J., Voisin, S., Herbert, A. J., Garton, F., Houweling, P. J., . . . Eynon, N. (2018). No association between ACTN3 R577X and ACE I/D polymorphisms and endurance running times in 698 Caucasian athletes. *BMC Genomics*, 19(1). <https://doi.org/10.1186/s12864-017-4412-0>
- Papadimitriou, I. D., Lucia, A., Pitsiladis, Y. P., Pushkarev, V. P., Dyatlov, D. A., Orekhov, E. F., . . . Eynon, N. (2016). ACTN3 R577X and ACE I/D gene variants influence performance in elite sprinters: a multi-cohort study. *BMC Genomics*, 17(1). <https://doi.org/10.1186/s12864-016-2462-3>
- Petro, J., Bonilla, DA. (2015). *Metabolismo Energético durante el Esfuerzo Físico: hacia una Conceptualización Actual en las Ciencias de la Actividad Física y el Deporte*. Paper presented at the XII Congreso Internacional en Ciencias de la Actividad Física y el Deporte, Tijuana, México.
- Pickering, C., Kiely, J., Grgic, J., Lucia, A., & Del Coso, J. (2019). Can Genetic Testing Identify Talent for Sport? *Genes*, 10(12), 972. <https://doi.org/10.3390/genes10120972>
- Ribas, M. R., Oliveira Netto, Z. C., Salgueirosa, F., Fernandes, P., Matos, O. d., & Bassan, J. C. (2017). Association of ACTN3 R577X and ACE I/D polymorphisms in Brazilians wrestlers. *Revista Brasileira de Medicina do Esporte*, 23, 469-472. <https://doi.org/10.1590/1517-869220172306171864>
- Rigat, B., Hubert, C., Alhenc-Gelas, F., Cambien, F., Corvol, P., & Soubrier, F. (1990). An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *Journal of Clinical Investigation*, 86(4), 1343-1346. <https://doi.org/10.1172/JCI114844>
- Sabir, J. S. M., Omri, A. E., Ali Khan, I., Banaganapalli, B., Hajrah, N. H., Zrelli, H., . . . Khan, M. (2019). ACE insertion/deletion genetic polymorphism, serum ACE levels and high dietary salt intake influence the risk of obesity development among the Saudi adult population. *Journal of the Renin-Angiotensin-Aldosterone System*, 20(3), 147032031987094. <https://doi.org/10.1177/1470320319870945>
- Sánchez, J., Campuzano, Ó., Iglesias, A., & Brugada, R. (2009). Genética y deporte. *Apunts. Medicina de l'Esport*, 44(162), 86-97. [https://doi.org/10.1016/S1886-6581\(09\)70114-8](https://doi.org/10.1016/S1886-6581(09)70114-8)
- Saunders, C. J., September, A. V., Xenophontos, S. L., Cariolou, M. A., Anastassiades, L. C., Noakes, T. D., & Collins, M. (2007). No Association of the ACTN3 Gene R577X Polymorphism with Endurance Performance in Ironman Triathlons. *Annals of Human Genetics*, 71(6), 777-781. <https://doi.org/10.1111/j.1469-1809.2006.00385.x>
- Stucky-Byler, L., Rodríguez-Buitrago, A. . (2018). *Análisis exploratorio de la relación entre el polimorfismo ACTN3 R577X y el rendimiento deportivo en levantadores de pesas colombianos* (Master's thesis), Universidad de Ciencias Aplicadas y Ambientales U.D.C.A., Retrieved from <https://repository.udca.edu.co/handle/11158/1056>
- Valderrama, A., Endo, M. . (2009). Frecuencia y distribución del polimorfismo I/D del gen ACE en adultos jóvenes de la ciudad de Cali. Paper presented at the 10th Colombian Conference of Human Genetics.
- Vallejo, D., Guerrero, M., Bejarano, L., Valderrama, A. (2009). Prevalencia de la Mutación R577X del Gen ACTN3 en Adultos Jóvenes de la Ciudad de Cali. Paper presented at the 10th Colombian Conference of Human Genetics.
- Vincent, B., De Bock, K., Ramaekers, M., Van den Eede, E., Van Leemputte, M., Hespel, P., & Thomis, M. A. (2007). ACTN3 (R577X) genotype is associated with fiber type distribution. *Physiological Genomics*, 32(1), 58-63. <https://doi.org/10.1152/physiolgenomics.00173.2007>
- Webborn, N., Williams, A., McNamee, M., Bouchard, C., Pitsiladis, Y., Ahmetov, I., . . . Wang, G. (2015). Direct-to-consumer genetic testing for predicting sports performance and talent identification:

- Consensus statement. *British Journal of Sports Medicine*, 49(23), 1486-1491. <https://doi.org/10.1136/bjsports-2015-095343>
- Woods, D., Hickman, M., Jamshidi, Y., Brull, D., Vassiliou, V., Jones, A., . . . Montgomery, H. (2001). Elite swimmers and the D allele of the ACE I/D polymorphism. *Human Genetics*, 108(3), 230-232. <https://doi.org/10.1007/s004390100466>
- World Medical Association. (2013). World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. *JAMA*; 310(20):2191–2194. <https://doi.org/10.1001/jama.2013.281053>
- Yang, N., MacArthur, D. G., Gulbin, J. P., Hahn, A. G., Beggs, A. H., Eastal, S., & North, K. (2003). ACTN3 Genotype Is Associated with Human Elite Athletic Performance. *The American Journal of Human Genetics*, 73(3), 627-631. <https://doi.org/10.1086/377590>
- Yang, R., Shen, X., Wang, Y., Voisin, S., Cai, G., Fu, Y., . . . Yan, X. (2017). ACTN3 R577X Gene Variant Is Associated With Muscle-Related Phenotypes in Elite Chinese Sprint/Power Athletes. *Journal of Strength and Conditioning Research*, 31(4), 1107-1115. <https://doi.org/10.1519/JSC.0000000000001558>
- Znazen, H., Mejri, A., Touhami, I., Chtara, M., Siala, H., Ahmetov, I., . . . Soussi, N. (2015). Genetic advantageous predisposition of angiotensin converting enzyme id polymorphism in Tunisian athletes. *J Sports Med Phys Fitness*, 56(6), 724-730.

