ACTN3 genotypes and their association with athletes somatotype: Results of a pilot study

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Abstract

The genotypes RR, RX and XX of the alpha-actinin-3 gene (ACTN3), are related to the physical-sports abilities and body composition, however, there were not found studies that relate the genotypes with the somatotype. The purpose of this study was to know if the ACTN3 genotypes are associated with the somatotype in athletes regardless of the sport they practice. In a cross sectional study 31 athletes from different sports were evaluated. DNA from white blood cells in peripheral blood were obtained. Anthropometric measurements were taken and so the fat mass, muscle mass, and somatotype were calculated. Both men and women, carriers of the RR genotype presented greater mesomorphy, followed by the RX genotype carriers. In the case of men, carriers of genotype XX presented greater ectomorphy. Men with RR and RX genotypes exhibit a mesomorph-balanced somatotype, women with the same genotypes, present an endomorph-mesomorphic somatotype. Men with genotype XX exhibit an ecto-mesomorphic genotype, females an endo-mesomorphic somatotype. The mesomorphy is the main component of the somatotype that defines the athletes carrying the RR genotype, while the ectomorphism to the XX genotype carriers.

Sports Genetics, Alpha-Actinin-3, Body Composition

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1. Introduction

The human body form is genetically determined, however, it can be modified by external factors generally referred to as environmental factors, such as physical activity and diet (Huygens et al., 2004). The somatotype developed by Sheldon (1954), is a representation of the body form, where the main components endo (fat), meso (muscle) and ectomorphy (linearity) characterize the subject. Johnson et al. (2015), reported that indicators of adiposity as percentage of fat and fat mass, express less variability to greater cardiovascular conditioning; that is, physical activity exerts in each subject a suppressive effect on the variability of fat tissue, caused by external factors. With regard to lean mass, its variability is influenced more by genetic factors than by environmental influences (Johnson et al., 2015).

Product of these effects, genetic and environmental, the dispersion in the endomorphic component is greater in sedentary subjects, than among athletes (Rivera-Sosa, 2016), when its somatotype is graphed in the somatocarta. On the other hand, in studies of heritability in the somatotype, it has been documented that mesomorphy is determined up to 59% by additive genetic factors, ectomorphy by 45% (Jelenkovic et al., 2011) and endomorphy by 40%. % (Saranga et al., 2008). Therefore, checking the influence of genes related to muscle function and their participation in the development of muscle mass, body composition or body shape is one of the areas to be investigated.

That is, to analyze whether the genotypes associated with aerobic and anaerobic capacity, strength or muscular endurance (Oliveira et al., 2016), are also involved in the corporal form. Recently, one of the most studied genes in the field of sports performance and physical abilities has been alpha-actinin-3 (ACTN3).

This gene has two alleles: the R allele, which functionally encodes the alpha-actinin-3 protein, while the X allele encodes it non-functionally (MacArthur and North, 2004). Alpha-actinin-3 is one of the main structural proteins of the sarcomere Z line at the muscle level and is expressed only in skeletal muscle fast fibers (Beggs et al., 1992). The genotypes of ACTN3 (RR, RX and XX) have been associated with sports performance: aerobic (Ahmetov et al., 2010; Pimenta et al., 2013) or anaerobic (Mikami et al., 2014; Papadimitriou et al. ., 2016). In this sense, Yang et al. (2003), reported that genotype XX favors aerobic capacity activities; while the RR genotype favors anaerobic capacity activities, such as the speed of movement and the generation of muscular strength.

The literature published in the main scientific bases, does not show up to the present, reports on the associations between the genotypes of ACTN3 and the somatotype. The athlete population represents a population group where the lower variability of the fat component could allow detecting the influence of the ACTN3 gene on the somatotype components. To establish if: 1) the carriers of the RR and RX genotypes vs. XX, present greater mesomorphy, and if 2) the carriers of the genotype XX vs. RR and RX, present greater ectomorphy. Therefore, the purpose of the present work was to determine the possible associations between the genotypes of ACTN3 and the somatotype in Mexican university athletes.

2. Materials and methods

Subjects

Under a cross-sectional design and for convenience, 31 athletes of competitive university and national level (18 men and 13 women) were recruited. The inclusion criteria were: male and female athletes participating in national and local competitive events, age 18 to 30 years.

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We excluded athletes with less than 2 years of practicing their sport, with the presence of any disease, who were under medical treatment or muscle injury. The athletes agreed to participate voluntarily in the study and signed the informed consent letter. The study was approved by the ethics committee of the Autonomous University of Ciudad Juárez, based on the recommendations of the Helsinki Declaration.

Genotypes

A blood sample was obtained through a puncture in the middle ulnar vein; subsequently, Genu Puregene Blood Kit commercial kit (Gentra, Minneapolis, USA) was used to obtain leukocyte genomic DNA. A segment of 291 base pairs (bp) of the ACTN3 gene was amplified using the polymerase chain reaction (PCR) technique, using the following primers: forward primer: 5'-CTGTTGCCTGTGGTAAGTGGG-3 ', on the other hand used the reverse primer: 5'-TGGTCACAGTATGCAGGAGGG-3 '. The following amplification conditions were applied: initial denaturation at 95 ° C for 10 min, followed by 35 cycles of denaturation at 95 ° C for 1 min, an alignment at 60 ° C for 30 s, elongation at 72 ° C for 1 min .

The PCR product was visualized on 1% agarose gels. For the determination of ACTN3 genotypes, the PCR amplification and the DdeI enzyme (Desulfovibrio desulfuricans) (BioLabs, Inc. Beverly, USA) were combined and incubated at 37 ° C in humid heat for 4 h, then the enzyme it was inactivated at 65 ° C for 20 min. The digestion products were observed in 2% agarose gels and visualized through a UV transilluminator (Bio-Rad, Hercules, USA). For the RR genotype the following bands were obtained: 205 bp and 86 bp, while for the RX genotype: 205 bp, 108 bp, 97 bp and 86 bp, finally, for the genotype XX: 108 bp, 97 bp and 86 pb.

Anthropometric measurements

All these measurements were made following the methodology standardized by the International Society for the Advancement of Cineanthropometry (ISAK for its acronym in English), registering the weight, height, 9 skinfolds, 13 perimeters, 10 lengths and 6 body diameters (Kevin and Olds, 1996). The weight was recorded in kilograms with a digital scale (SECA 876, Hamburg, Germany), taking care that the athlete's feet were in a central position and symmetrical on the scale, which was handled on a flat, horizontal and smooth surface.

The size was recorded in centimeters using a stadiometer (SECA 206, Hamburg, Germany), with an approximation of 1 mm. For the stature it was taken care of that the person was barefoot, the feet together and kept his head in the Frankfort plane; this measurement was recorded at the end of a deep inhalation, asking the person to maintain a right posture during the measurements. The BMI was calculated by weight / height2. For the other measurements an anthropometric equipment was used (Rosscraft Tom Kit, Surrey, Canada). The software LifeSize 2.0 (Lifesize 2.0, Sydney, Australia) was used to determine the components of the somatotype (Olds and Norton, 2000).

Statistic analysis

To determine the differences in the genotypic frequencies, a Chi square was performed (χ^2). To find differences between genotypes and between each variable studied, a one-way ANOVA was performed with the Tukey post hoc test. To find differences between genotypes and between sex, a two-way ANOVA was performed. To know the independent participation of the genotype (RR, RX and XX) and sex (independent variables) in the determination of endomorphy and muscle mass (dependent variables) of the athletes, a multiple regression analysis was performed by the method of successive steps.

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A level of significance of 0.05 was established. The data was analyzed with the SPSS 22.0 program**.**

3. Results

The athletes practiced the following sports: speed and throws (n = 11), weightlifting (n = 1), physical-bodybuilding (n = 3), swimming (n = 1), soccer (n = 3), judo (n = 1), baseball (n = 5) and handball (n = 6).

The frequency of ACTN3 genotypes is shown in Table 1. The total population is in Hardy-Weinberg equilibrium (X2 = 0.00, P = 0.97). In the present study, it is shown that in both sexes, the carriers with RR genotype are more mesomorphic than the XX. Men with RR and RX genotypes exhibit a mesomorfo-balanced somatotype, whereas women with the same genotypes have an endomorph-mesomorphic somatotype. Men with genotype XX exhibit an ectomorphic-mesomorphic genotype, and women an endo-mesomorphic somatotype (Figure 1). The men compared with the women had greater muscle mass and greater ectomorphy, on the contrary lower fat mass and lower endomorphy (P <0.05) (Table 2).

Genotype and sex independently determined 41.4% of the differences in mesomorphy (R^2 = 0.41, P < 0.01).

Mesomorphy = 4.9 + (0.86 x sex) - (0.73 x genotype). For sex, 0 = women and 1 = men. For the genotypes, 1 = RR, 2 = RX and 3 = XX.

Sex independently determined 59.2% of the differences in the percentage of muscle mass (R^2 = 0.59, P < 0.01). The genotype was excluded in the model.

Muscle mass (%) = 37.2 + (8.8 x sex). Where 0 = women, 1 = men.

4. Discussion

The present study groups the athletes by their genotype and analyzes their somatotype regardless of the sport they were practicing. It was found in both genders that, the mesomorphy is higher in the athletes with the RR genotype, followed by the RX genotype. In contrast, ectomorphy was greater in athletes with the XX genotype. The above, agrees with what reported by Yang et al. (2003); Pimenta et al. (2013); Orysiak et al. (2014), who report that RR and RX genotypes are associated with an improvement in anaerobic sport performance.

In this same sense, genotype XX has been associated with a possible improvement in performance in sports activities with aerobic characteristics (Ahmetov et al., 2010, Pimenta et al., 2013); Athletes who practice these sports usually have a more ecto-mesomorphic somatotype (Bale et al., 1986). The population group of athletes practiced sports of both aerobic and anaerobic characteristics. In them, the training promotes optimal muscle development and minimizes endomorphic variability, which independent athletes could develop (Johnson et al., 2015). Normally, anaerobic-type athletes have greater muscle mass than aerobic-type athletes, indicating a greater mesomorphy (Poblano-Alcalá and Braun-Zawosnik, 2014).

The development of muscle mass associated with the genotype could be attributed to the fact that the RR and RX genotypes vs. XX have higher concentrations of testosterone (Ahmetov et al., 2014). This hormone has anabolic effects, increasing or promoting the development of muscle mass. In the same sense, it has recently been reported that the possessors of the RR vs. XX genotypes, their muscle fibers IIa and IIx have a greater cross sectional area (Broos et al., 2016), which allows them to develop greater power (Orysiak and col., 2014), strength (Broos et al., 2015) and speed (Mikami et al., 2014), aspects related to mesomorphy.

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In contrast, carriers of genotype XX have a higher proportion of slow fibers type I, compared to the genotype RR and RX (Ahmetov et al., 2011), favoring aerobic endurance activities (Yang et al., 2003). Among the athletes studied, a genotype distribution of 35% was found for the RR genotype, similar to that reported in Spanish swimmers (Ruiz et al., 2013), and in Israeli athletes (Eynon et al., 2009). For allelic distribution, 41% was found in the X allele, similar to what was previously reported in the Hispanic and European population (Mills et al., 2001), as well as in elite Lithuanian athletes (Ginevičiene et al., 2011).

The similarity in the allelic and genotypic frequencies with the European population, could be due in part to crosses between indigenous people with Spaniards, occurred during and after the Spanish conquest (Rangel-Villalobos et al., 2009), or to the high conservation of the ACTN3 gene that it has remained in the population for a long time (Mills et al., 2001, MacArthur and North, 2004).

The allelic frequencies of this gene (R and X) and its genotype (RR, RX and XX) are very different among the population (Mills et al., 2001). In this sense, Yang et al. (2007), have found high prevalences of the RR genotype in African black athletes (40% to 87%), however, low prevalences in white athletes (17% to 30%). Regarding the genotype XX, Yang et al. (2007), found very low or absent prevalences in African black athletes (0% to 12%), whereas low prevalences in white athletes (18% to 25%).

The results of this study, although they were not done with a population interest, are the first report of its prevalence in a Mexican population of athletes at the university level. On the other hand, it was also obtained that besides having sex, having a certain genotype favors partially, also the presence of a certain somatotype (41% of the variance observed).

To our knowledge, this is the first work that reports an association between genotypes of the ACTN3 gene and the somatotype. However, being a pilot study, the results obtained need confirmation in a larger study.

5. Conclusions

As a main result, it was observed that mesomorphy is the main component of the somatotype that defines the athletes carrying the RR genotype, however, ectomorphy is the main component of the somatotype that defines the carriers of the XX genotype. Between 41% and 59.2% of differences found in mesomorphy and ectomorphy were determined by differences in genotype and sex

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Anexo

Sample	Genotypic frequency n (%)			Allelic frequency %	
	RR	RX	XX	R	X
Total athletes (31)	11 (35.6)	15 (48.1)	5 (16.2)	59.7	40.3
Men (18)	7 (37.3)	8 (47.5)	3 (15.1)	61.1	38.9
Women (13)	4 (33.2)	7 (48.8)	2 (17.9)	57.7	42.3
χ^2= 0.00; P = 0.97.					

Table 1 Genotypic and allelic frequencies of the ACTN3 gene

	Men			Women		
	RR (n= 7)	RX (n= 8)	XX (n= 3)	RR (n= 4)	RX (n= 7)	XX (n= 2)
Weight (kg)	77.7 ± 8.4	71.3 ± 11.4	72.10 ± 6.2	60.9 ± 6.1	63.5 ± 12.8	72.6 ± 8.8
Height (m)	1.81 ± 0.03	1.76 ± 0.05	1.83 ± 0.09	1.61 ± 0.08 [a]	1.66 ± 0.09[a]	1.71 ± 0.02[a]
BMI (kg / m2)	23.7 ± 2.2	23.0 ± 2.8	21.5 ± 0.8	23.6 ± 0.8	23.0 ± 2.7	24.8 ± 3.5
Body fat (%)	14.4 ± 7.2	11.5 ± 3.9	9.2 ± 2.4	16.4 ± 4.0[a]	16.2 ± 4.0[a]	19.1 ± 2.3[a]
Muscle mass (%)	48.8 ± 4.2	44.2 ± 3.0	44.7 ± 1.7	37.4 ± 3.9[a]	37.6 ± 3.9[a]	35.6 ± 2.0[a]
Endomorphy	2.9 ± 1.5	2.7 ± 1.4	1.9 ± 0.4	4.2 ± 1.1[a]	3.7 ± 1.2[a]	4.8 ± 1.1[a]
Mesomorphy	5.0 ± 0.7	4.6 ± 1.0	3.1 ± 1.1[b]	4.2 ± 0.4	3.3 ± 0.9	3.2 ± 0.9[b]
Ectomorphy	2.5 ± 1.0	2.7 ± 1.1	3.6 ± 0.8	1.4 ± 0.6[a]	2.0 ± 1.0[a]	1.6 ± 1.3[a]
Values are presented in averages ± DE. [a] differences between sex. [b] differences between genotype RR *vs* XX. P < 0.05.						

Table 2 Anthropometric characteristics between sex and genotypes of ACTN3

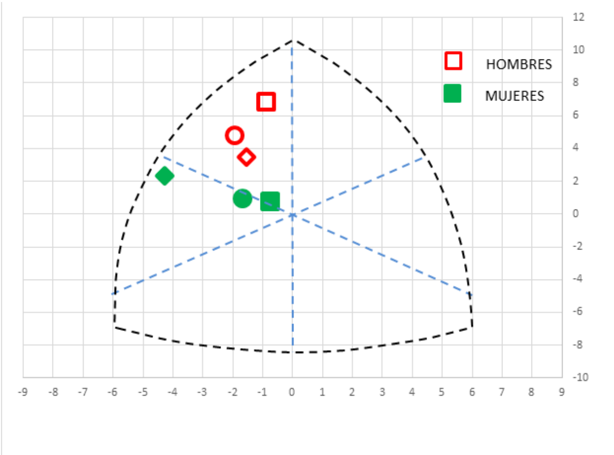

Figure 1 Average somatotype in men and women athletes with different genotypes of ACTN3. Figures without filling correspond to men, figures with fill correspond to women. Squares: RR genotype, Circles: RX genotype, Rhombos: genotype XX. Significant difference was found in the carriers of the RR vs. XX genotype ($P < 0.05$).