



ACTN3 R577X and ACE I/D Genotype Frequencies of Professional Soccer Players in Brazil

Fabiano de Macedo Salgueirosa^{1,5}, Patrick Rodrigues^{2,5}, Gerusa Gabriele Seniski³, Lee Wharton⁴, Raul Osiecki⁵

¹University Positivo, Curitiba, Brazil, ²Univali, Itajaí, Brazil;
³UniBrasil, Curitiba, Brazil, ⁴Faculty of Health, School of Exercise and Nutrition Sciences, Queensland University of Technology, Brisbane, Australia, ⁵Center of Study of Physical Performance, Federal University of Paraná, Curitiba, Brazil

ABSTRACT

Salgueirosa FM, Rodrigues P, Seniski GG, Wharton L, Osiecki R. ACTN3 R577X and ACE I/D Genotype Frequencies of Professional Soccer Players in Brazil. **JEPonline** 2017;20(6):129-138. The purpose of this study was to identify the genotype and allele distribution of ACTN3 and ACE genes in professional soccer players and to compare its distribution to the general population. Forty male professional players of a Brazilian first division soccer team participated in this study. The subjects' blood samples were drawn from an antecubital arm vein. Genomic DNA was extracted from leukocytes using the salting-out technique. ACTN3 and ACE polymorphism was determined by the polymerase chain reaction (PCR). With regards to the ACTN3 R3557X gene of soccer player frequency distributions, the genotype RX(50%)>RR(45%)>XX(5%) and the allele R(70%)>X(30%). As to the ACE I/D gene distribution, the genotype ID(57.5%)>DD(30%)>II(12.5%) and allele D(58.8%)>I(41.2%). The soccer player genotype and allele frequencies were not different from the control groups. In conclusion, our data indicate that ACTN3 R577X and ACE I/D are not the best genetic marker for identifying a talented soccer player. Nevertheless, these results suggest that men who have the combination of both genotypes 'ACTN3 RX' and 'ACE ID' in these two different genes are more likely to be a professional soccer player when compared to other genotype combinations.

Key Words: ACTN3, ACE, Genetics, Genotype, Soccer

INTRODUCTION

High performance results are based on the combination of environmental factors, such as, suitable training loads and nutrition. However, such factors are not able to explain the large variation in training responses. This knowledge presents a new important component for determining the phenotype of physical performance, that being, the genetic component (10). The concept of human variation in the ability to respond to training was proposed three decades ago, and since then several studies conducted with monozygotic twins have confirmed a substantial genetic component in response to training (3).

A change in the DNA base sequences (polymorphisms) may influence the expression and activity of certain proteins and, therefore, in number of forms involved in the variation of the physical performance phenotype. It is believed that ~200 genetic variations (candidate genes) are related to phenotypes of physical performance, fitness, and health (4). Among the candidate genes studied the gene encoding α -actinin 3 (ACTN3) and the gene encoding the angiotensin-converting enzyme (ACE) have been shown to be promising in studies that have a focus on training responses (22). In addition, over the last two decades, many sport science studies have been conducted to investigate the relationship between genetics and elite athletic performance (17). It is expected that with the rapid development of gene-based technologies, further research will be carried out in order to identify genetic predispositions as a contributing factor to athletic abilities and performance (25).

The ACTN3 R577X polymorphism promotes an arginine (R) to premature stop codon (X) change in the 577 residue (23), resulting in a complete absence of the protein α -actinin-3 in type II muscle fibres in individuals carrying the XX genotype. This absence might have some consequences. It has been demonstrated that α -actinin-3 absence induces metabolic changes towards oxidative metabolism, resulting in higher activity of oxidative enzymes (e.g., citrate synthase) and lower activity of glycolytic enzymes (e.g., lactate dehydrogenase and glycogen phosphorylase) (27). Because of this, it has been observed that XX individuals are over-represented among long-distance athletes (34). It has also been observed that the presence of α -actinin-3 leads to an increased force generation, increased fast fiber diameter and higher strength capacity (18). Hence, RR individuals are over-represented among power-oriented athletes (11). These results indicate that X and R alleles of the ACTN3 R577X polymorphism could lead to opposite phenotypes resulting in specific advantages in activities presenting distinct characteristics.

To date, ACE gene is one of the most studied genetic polymorphisms that has been shown to be associated with athletic performance (17). The product of ACE is one of the renin angiotensin system (RAS), which catalyzes the production of angiotensin II from angiotensin I, resultantly increasing blood pressure (7). Moreover, the ACE is expressed in skeletal muscle, where it influences its function and biomechanical properties (12,15). The ACE human gene is located on chromosome 17 in position 17q23.3 with a restriction fragment length polymorphism consisting of the presence (insertion, I) or absence (deletion, D) of a 287 base pair Alu repeat sequence in intron 16 (28). In this case, three ACE genotypes include DD, II homozygotes, and ID heterozygotes. According to Cięszczyk et al. (7), studies concerning associations between the ACE genotype and athlete status have shown that I allele is associated with lower ACE activity in both serum and tissue and improved performance in endurance sports, while the D allele is associated with greater circulating and

the ACE activity and causes an enhanced performance in sports requiring sprinting or muscle power.

Soccer is a team based sport and from a metabolic point of view is considered to be a mixed modal sport. The average distance travelled by a high-level athlete is around 10 to 13 km per game (2) with intensities close to the anaerobic threshold (14) coupled with intermittent efforts and intensity changes every 4 to 6 sec (21). Despite being a predominantly aerobic sport, decisive actions are characterized by moments of short duration and high intensity with predominance of anaerobic metabolism. Several moments of explosive activity are needed, such as jumps, kicks, sprints, and changes of direction (32). According to Mohr et al. (21) elite soccer players perform between 150 and 250 intense actions during a single match. These actions indicate that the anaerobic energy utilization rate is high at certain times of the game (2), making the muscle power and strength crucial in many game situations (6).

Given the aforementioned context, the sport of soccer involves multiple characteristics of performance that includes metabolic (aerobic, anaerobic, and intermittent components) and neuromuscular parameter (power, strength, sprint, jumps, and change directions). The knowledge of the distribution of each genotype that plays a specific role on these performance features offers the technical staff important information concerning the personalized treatment of training loads, recovery periods, and precise stimulus of skilled training. Furthermore, the comparison of genotype and allele distribution to the general population may help to clarify whether ACTN3 and ACE genes can be used in talent identification and selection of elite soccer players. Thus, this study aimed to identify the genotype and allele distribution of ACTN3 and ACE genes in professional soccer players and to compare its distribution to the general population.

METHODS

Subjects

Forty male professional players of a Brazilian first division soccer team (mean $\pm$ SD; age: 24.57 ± 4.35 yrs; height: 179.15 ± 7.49 cm; weight: 77.17 ± 8.06 kg; body fat: 11.66 ± 2.81 %; VO_2 max: 65.37 ± 3.55 mL $\cdot$ kg $^{-1}\cdot$ min $^{-1}$) that maintained regular training sessions and competed in official events organized by the Brazilian Soccer Federation participated in this study. This study was approved by the Research Ethics Committee of the Department of the Health Sciences of the Dom Bosco University (protocol: 225.747), and it is in compliance with all the norms established by the National Health Council (Res. 196/96) regarding research with human subjects. Following the subjects receiving a comprehensive verbal and written explanation about the benefits and risks of the study, they signed the informed consent document that was institutionally approved.

The soccer players' ACTN3 R577X genotype distributions in this study were compared with the general population distribution of the Silva et al. (31) study. They used 206 healthy Brazilian males (mean $\pm$ SD; age: 25.33 ± 3.67 yrs; height: 175.66 ± 0.01 cm; weight: 75.30 ± 9.57 kg; body mass index: 24.33 ± 2.80 (kg $\cdot$ m $^{-2}$); VO_2 max: 29.67 ± 8.07 mL $\cdot$ kg $^{-1}\cdot$ min $^{-1}$). The subjects in the present study were not engaged in regular physical activity for at least 6 months.

The studies of Meira-Lima et al. (19) and Pardono (24) were used to compare the ACE I/D genotype distributions. Both studies used Brazilian subjects. Meira-Lima and colleagues evaluated 323 healthy subjects (mean $\pm$ SD; age: 32.00 ± 9.60 yrs; 50% males and 50% females (the general population), while Pardono evaluated 62 physically active men with good aerobic condition, but not professional soccer players (mean $\pm$ SD; age: 23.30 ± 4.20 yrs; height: 177.70 ± 5.50 cm; weight: 75.50 ± 9.80 kg; body fat: $12.00 \pm 4.60\%$; body mass index: 23.90 ± 2.50 ($\text{kg} \cdot \text{m}^{-2}$); VO_2 max: 50.20 ± 6.20 $\text{mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$).

Procedures

Sample Collection and DNA Extraction

Blood samples of the soccer players were drawn from an antecubital arm vein using a 20-gauge disposable needle equipped with a Vacutainer tube holder (Becton Dickinson, Franklin Lakes, NJ). The collected samples (10 mL) were held in Vacutainer tubes containing SST-Gel and a Clot Activator. The samples in tubes were then stored under refrigeration (2° to 8° C) for a maximum of 7 days until the DNA extraction. Genomic DNA was extracted from leukocytes in samples of whole blood using the salting-out technique (kit: BioPur Spin 50 - Biometrix, Curitiba) as recommended by the manufacturer's instructions.

ACTN3 R577X and ACE I/D Single Nucleotide Polymorphism Genotyping

A DNA fragment carrying the exon 16 from the ACTN3 gene R577X polymorphism was amplified from the genomic DNA and the following initiators were used: 5'-CTGTTGCCTGTGGTAAGTGGG-3'; reverse, 5'-TGGTCACAGTATGCAGGAGGG-3', correlated to the adjacent intronic sequences (20). The polymerase chain reaction (PCR) analysis was used in the same manner as in Pimenta et al. (26). The amplification program consisted of an initial denaturation at 94°C for 5 min, followed by 30 cycles, comprising 94°C for 1 min, 64°C for 1 min, and 72°C for 1 min with a final extension of 72°C for 5 min. The R577X alleles (codons CGA and TGA) were distinguished by the presence (577X) or absence (577R) of a restriction site of the enzyme DdeI (20). The ACTN3 577R allele generates fragments in 205 and 86 base pairs (bp), while the ACTN3 577X allele generates fragments in 108, 97, and 86 (34).

ACE gene I/D polymorphism was determined by PCR conditions that were as follows: initial denaturing at 95°C for 5 min; 35 cycles at 95°C for 30 sec, 58°C for 30 sec, 72°C for 1 min, and a final extension at 72°C for 5 min. Using a primer pair flanking the polymorphic region of intron 16 that produces either an amplified 490bp (I allele) or a 190bp product (D allele), or both. The intron 16 was amplified using initiators: 5'-CTGGAGAGCCACTCCCATCCTTTCT-3'; reverse, 5'-GATGTGGCCATCACATTCGTCAGAT-3'. The reactions were performed in accordance with Rigat et al. (28). The possibility of mistyping I/D heterozygotes as DD homozygotes due to preferential amplification of the smaller D allele was addressed by further amplification using a primer pair that recognized insertion-specific sequence (30).

Statistical Analyses

SPSS 20.0 for Windows (SPSS, Inc., New York, USA) was used for the data analysis. The distribution of the ACTN3 and ECA genotypes was tested to determine if it was in Hardy-Weinberg equilibrium by using a chi-square test. The distributions of the ACTN3 alleles and genotypes for the general population and the soccer players, and the ECA alleles and

genotypes for the general population, physically active subjects and soccer players were compared using chi-square tests. The level of significance was set at 0.05.

RESULTS

The ACTN3 R577X and ACE I/D genotype frequencies met Hardy-Weinberg expectation in the soccer player subjects, with P-Value = 0.2283 and P = 0.2396, respectively for the genes. The distribution of the ACTN3 genotypes and alleles of the professional Brazilian soccer players and the general population are summarized in Table 1. The distribution of ACE genotypes and alleles of the soccer players, physically active men, and the general population are presented in Table 2. When the frequencies of the ACTN3 and ACE genotypes of soccer players were compared with the general population or physically active subjects in a single test for each comparison, no significant association was found in both genes ($P > 0.05$).

Table 1. Frequencies of ACTN3 Genotypes among the General Population and Soccer Players.

	RR	RX	XX	R	X
	N(%)	N(%)	N(%)	N(%)	N(%)
General Population	76(36.9)	97(47.1)	33(16)	249(60.4)	163(39.6)
Soccer Players	18(45)	20(50)	2(5)	56(70)	24(30)

Values shown as absolute frequencies (relative frequencies in parentheses). Comparison between general population genotype frequencies (SILVA et al. 2015) and soccer player genotype frequencies (present study), $P = 0.331$.

Table 2. Frequencies of ACE Genotypes among the General Population, Physically Active Subjects and Soccer Players.

	DD	ID	II	D	I
	N(%)	N(%)	N(%)	N(%)	N(%)
General Population	104(32.2)	155(47.9)	64(19.9)	363(56.2)	283(43.8)
Physically Active Subjects	16(25.8)	31(50)	15(24.2)	63(50.8)	61(49.2)
Soccer Players	12(30)	23(57.5)	5(12.5)	47(58.8)	33(41.2)

Values shown as absolute frequencies (relative frequencies in parentheses). Comparison between general population genotype frequencies (MEIRA-LIMA et al. 2000) and soccer player genotype frequencies (present study), $P = 0.914$. Comparison between physically active subjects genotype frequencies (PARDONO, 2010) and soccer player genotype frequencies (present study), $P = 0.707$.

DISCUSSION

The purpose of this study was to compare the genotype distribution of ACTN3 and ACE genes of Brazilian soccer players with a control group (general population and physically active men). We hypothesized that the ACTN3 and ACE genes in Brazilian soccer player would present different frequency distribution than our control groups; however, no differences were found. The frequencies of ACTN3 R577X genotypes and alleles were no different between Brazilian soccer players and the general population, nor even the ACE I/D polymorphism in soccer player genotype and allele frequencies when compared to the general population or physically active men.

The ACTN3 RR and RX genotypes have been associated with high speed and strength athletes (18,33), while XX genotype has been associated with aerobic endurance performance (1,5). Furthermore, Yang et al. (34) identified a higher frequency of ACTN3 RR genotype and allele R in professional athletes enrolled in modalities that require physical strength and power capacities. On the other hand, ACTN3 XX genotype and X allele are more frequently associated with athletes involved in high endurance performance tasks. The fact of soccer is considered to be a mixed modal sport, with anaerobic, aerobic, and intermittent efforts performed every game, it may explain why the ACTN3 genotype frequencies of soccer players shows a more homogeneous distribution than other sports and did not present as different to that of the general population in this study. Similarly, other studies have also failed to differentiate between professional soccer players and the general population in Brazil (9) and Spain (29). Considering the potential benefits of the R allele for power and sprint athletic performance, and the X allele for endurance athletic performance (17), it would be biologically logical to consider that both alleles (R and X) may play a key role in identifying and/or developing soccer players where both power and endurance phenotypes are important.

Despite the present study not establishing a statistical differences in ACTN3 frequency distributions, the RR genotype and R allele were found to be more frequent in soccer players than in individuals in the control group (RR: 45% vs. 36.9; R: 70% vs. 60.4%, respectively). Conversely, the XX genotype and X allele showed lower frequencies in soccer players (XX: 5% vs. 16%; X: 30% vs. 39.6%, respectively) (Table 1). These data suggest a tendency that ACTN3 577R may be an indicator of natural selection for mixed modal sports such as soccer. Men with R allele would be more likely to become an elite soccer player in terms of performance than subjects with X allele; however, their personal skills are also very important to this type of sport. According to Coelho et al. (8), in team sports such as soccer, it is not only physical condition that affects high performance but also tactics and the technique of each individual.

ACE genotype and athlete status have shown that I allele is associated with lower ACE activity in both serum and tissue and improved performance in endurance sports while the D allele is associated with greater circulation and the ACE activity and causes an enhance performance at sports requiring sprinting or short bursts of power (7). Moreover, Zhang et al. (35) analyzed the muscle biopsy of 41 untrained, healthy young volunteers and found an association of II genotype with type I skeletal muscle fiber and DD genotype with type IIb skeletal muscle fiber. In the present research, the frequency of soccer players ACE I/D genotypes were compared to two different studies as control, the first study used a high number of subjects (N = 323), but 50% were males and 50% females (the general population)

(19), and the second study used a lower number of subjects (N = 62), but all subjects were males (physically active subjects) (24).

No differences were found between the ACE genotype frequency of soccer players to the general population or physically active men groups (Table 2). Similarly, another study (16) did not find any differences between ACE genotype and allele frequencies of Spanish soccer players and a control group. But, the researchers did find differences when the soccer players were compared with long-term runners (52 Spanish Olympic class males). The ID genotype frequency distribution of soccer players was higher than that of runners and II genotype distribution was also lower.

In our data, the ACE DD and II genotype frequencies of soccer players were lower than general population (2.2% and 7.4%, respectively), but the ID genotype frequency of soccer players was 9.6% higher. This may confirm that, the combinations of physical phenotypes of both alleles (D and I) that have shown different characteristics of performance are important for soccer players. When the ACE genotype frequencies of soccer players were compared to physically active men with good aerobic condition who participated in sports other than soccer, the DD genotype frequency was a bit higher (4.2%) and the II was lower (11.7%). These findings are similar to the study of Juffer et al. (16), where the I allele was less frequent in soccer players when compared to the subjects of other sports with good aerobic condition.

According to Pimenta et al. (26), although soccer is considered as a long-duration exercise, it is well known that matches are won in high short-effort durations (sprinting or jumping). Therefore, besides technical and tactical skills, muscular strength and “explosive” leg power are the most important factors contributing to successful performance during elite soccer competitions. Even without statistical differences, the finding in our study showed higher frequency of genotypes that have a association with power and strength than genotypes associated with endurance (ACTN3 RR 45% vs. XX 5%; ACE DD 30% vs. II 12.5%). This alone reinforces the claims of the aforementioned authors (26). Nevertheless, our study observed that the genotypes that present both alleles (ACTN3 RX 50%; ACE ID 57.5%) are predominantly identified in soccer players’ genotype frequency distributions.

CONCLUSIONS

Regarding the gene ACTN3 R3557X frequency distributions in Brazilian elite soccer players, the genotype RX>RR>XX and the allele R>X and the gene ACE I/D distribution, the genotype ID>DD>II and allele D>I. Theses frequencies were not different from the control groups, indicating that ACTN3 R577X and ACE I/D are not the best genetic markers for identifying a talented soccer player. Nevertheless, our results suggest that men who have a combination of both genotypes ‘ACTN3 RX’ and ‘ACE ID’ in these two different genes are more likely to be a professional soccer player when compared to other genotype combinations.

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Address for correspondence: Patrick Rodrigues, 250, João Fernandes Vieira Junior St, Itajaí, Santa Catarina, Brazil, Zip: 88302-600. Email: prof.patrickrodrigues@yahoo.com.br

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