Evidence for a Role of ACTN3 R577X Polymorphism in Football Player’s Career Progression

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Abstract
The aim was to investigate a possible role of the ACTN3 R577X polymorphism in a Brazilian football player’s career progression. 2 questions were formulated: 1. Does ACTN3 polymorphism affect the probability of an individual being a professional football player? 2. Does this polymorphism affect the progression of the athlete throughout his career? The study included 353 players from first division Brazilian football clubs in the following categories: under-14 (U-14), U-15, U-17, U-20, and professional (PRO). The control group (CON) was composed of 100 healthy non-athletes. The chi-squared test was used to assess differences between the allele and genotype frequencies. Comparing football categories, the XX genotype was less frequent among professional players than in the U-20 (p < 0.05) or the U-15 category (p < 0.05). The RX genotype also presented more frequently in the PRO category than the U-14 category (p < 0.05). Moreover, a trend towards a higher frequency of the RX genotype and a lower frequency of the XX genotype was observed in the professional category compared to U-20. These results suggest that the genotype in the ACTN3 polymorphism affects the probability of a football player progressing throughout his career and becoming professional, meaning that playing football selects against the ACTN3 XX genotype.

Introduction
Football is an activity with a global audience, and people of both sexes and all ages all around the world practice it [12]. During a football game, players cover a distance between 8 km and 12 km in a variety of ways, from walking to running at full speed [2]. The predominant mode of energy expenditure is aerobic [7], due to the long duration of the game; however, the actions that decide the result are anaerobic: sprints, jumps, headers, challenges, and shots on goal [38]. A competitive season presents a high volume of games over the years with little time for recovery between the competitions, producing a high physiological demand on the players [8].

Due to these characteristics, football can be classified as an intermittent, high-intensity activity. Thus, besides having good aerobic conditioning, football players must also possess great strength and speed, especially at high-performance levels [24]. It should be noted that these physical capacities, strength and power, are determined by genetic inheritance [40].

In athletes, it has been observed that the higher the competitive level, the greater the contribution of those genes related to the characteristics of the discipline (strength or endurance) [32, 41]. Other studies, however, have found no relationship between physical aptitude and the expression of genes related to those capabilities [35, 42].
The ACTN3 gene encodes alpha-actinin-3. This intrasarcomeric protein, specific to type II muscle fibers, plays a structural role in the arrangement and transfer of traction between actin filaments and the Z-line [3,6].

Alpha-actinin-3 has been associated with alterations of the contractile properties of the sarcomere in type II muscle fibers [16], effects on the differentiation of muscle fiber type or hypertrophy through indirect interactions between alpha-actinin-3 and signaling proteins such as calcineurin [37], alterations of the ability to resist and/or recover from muscle damage caused by exercise [27], changes to the metabolic profile of muscle fibers through altered interactions with metabolic enzymes [18], and improved glycogen consumption or signaling for muscle hypertrophy [25]. None of these scenarios is exclusive of any other, and the actual mechanism is likely to be a combination of several of these processes.

The genotypes expressing alpha-actinin-3 (ACTN3-RR and RX) have been linked to activities in which strength and speed are predominant [3,16,28,29,41]. The genotype that does not express alpha-actinin-3 (ACTN3-XX) has been linked to activities with a predominance of aerobic endurance [1,4,5,13,35]. Nonetheless, a recent multi-cohort quantitative analysis demonstrated that it is unlikely that the ACTN3-XX genotype provides an advantage in competitive endurance running performance [27].

Although the different ACTN3 genotypes have been studied in relation to intermittent collective activities [11,15], including football [16,30,34], the genetic profile of Brazilian football players at different competitive age groups has not yet been analyzed, especially in the Brazilian population of athletes, whose ethnicity mix differs from that in European countries [39]. An analysis of the genotypic frequency at different ages of football players may suggest how the ACTN3 interferes in the selection of athletes for football.

Measuring the distribution of genotype frequencies in football could contribute to the individualization of training loads [3,16,31]. Different aptitudes in relation to the expression of ACTN3 have also been observed among football players; ACTN3-RR athletes performed better in field tests related to strength and power [16], whereas ACTN3-XX individuals performed better on endurance tests [30]. This relationship was not always found [9,20]. These studies suggest that identifying the genetic profile of athletes, as performed in the present study, should be considered a benefit to the sports environment, especially because recent scientific evidence supports that the ACTN3 R577X polymorphism is associated with the incidence and severity of muscle injuries in professional football players. Players with the ACTN3 577XX genotype have higher odds of having muscle injuries than their RR counterparts [21].

Hence, in the present study, we aimed to identify the frequency of the ACTN3 polymorphism genotypes in Brazilian football players of different ages and study the role of ACTN3 R577X polymorphism in a football player’s career progression.

Materials and Methods

Ethical considerations

This study was approved by the Ethics in Research Committee of the Universidade Federal de Minas Gerais (ETIC-291/09) and complied with all the norms established by the Brazilian National Health Council (Resolution 466/2012) regarding research with human beings. All procedures, possible risks and benefits of the study were explained to the volunteers and their representatives before they signed the informed written consent to participate in the experiment. In addition, this study meets the ethical standards of the journal [14].

Participants

The sample of the present study included 353 male players (▶ Table 1) from 4 football clubs. These players belonged to the under-14 (U-14, up to 14 years old); under-15 (U-15, up to 15 years old); under-17 (U-17, 15 up to 17 years old); under-20 (U-20, 17 to 20 years old); and professional (PRO, players of the main team) categories. The ethnic backgrounds of the participants were different, hence determining the origin of these volunteers accurately was complex, but all were guaranteed to be descendants of Brazilian families up to at least the second generation. The subjects played in first-division Brazilian football clubs that had regular, systematic training sessions and took part in competitions organized and/or recognized by the Brazilian Football Confederation. These players had at least 2 years of competitive football experience and, when not playing in competitions, they trained an average of 6 days a week. Each training session lasted approximately 2 h, and they had about 9 training sessions per week. Some of these players were part of the Brazilian team that won the gold medal at the Olympic Games in Rio de Janeiro in 2016.

Allele and genotype frequencies for the ACTN3 R577X polymorphism in the population were also established. A group of 100 individuals (50% male and 50% female, healthy, physically active, aged 8–14 years) served as a control. This group was randomly selected and representative of the school population in the city of Belo Horizonte, Minas Gerais, Brazil. The sample of the control group was intended to represent the frequency of ACTN3 in the Brazilian population as a whole, even though male players composed the sample of the present study.

▶ Table 1 General characteristics of the sample according to categories.

<table>
<thead>
<tr>
<th>Category (N)</th>
<th>Age (years)</th>
<th>Weight (Kg)</th>
<th>Height (cm)</th>
<th>Body Fat (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>U-14 (43)</td>
<td>14.23 ± 0.32</td>
<td>69.12 ± 7.53</td>
<td>178.21 ± 9.72</td>
<td>10.43 ± 3.51</td>
</tr>
<tr>
<td>U-15 (68)</td>
<td>15.50 ± 0.50</td>
<td>68.42 ± 9.42</td>
<td>177.57 ± 8.52</td>
<td>9.49 ± 2.00</td>
</tr>
<tr>
<td>U-17 (44)</td>
<td>16.32 ± 0.25</td>
<td>73.30 ± 6.79</td>
<td>181.36 ± 7.04</td>
<td>9.15 ± 1.89</td>
</tr>
<tr>
<td>U-20 (115)</td>
<td>18.20 ± 0.40</td>
<td>73.36 ± 7.90</td>
<td>180.65 ± 8.20</td>
<td>9.21 ± 2.08</td>
</tr>
<tr>
<td>PRO (83)</td>
<td>23.50 ± 3.64</td>
<td>74.25 ± 5.79</td>
<td>182.60 ± 8.55</td>
<td>8.20 ± 2.34</td>
</tr>
</tbody>
</table>

Categories: under-14 (U-14), under-15 (U-15), under-17 (U-17), under-20 (U-20) and professional (PRO). Mean and standard deviation data.
Genotyping of the R577X polymorphism in the ACTN3 gene

Genomic DNA was extracted from the peripheral blood using a salting out/proteinase K procedure [22]. All data were collected between March and November 2016. Genotyping was performed using PCR-RFLP as described elsewhere [31]. Briefly, a DNA fragment covering the exon 16 from the ACTN3 gene was PCR-amplified using the primers ACTN3-for: 5'-CTG CCT GTG GTA ACT GGG-3' and rACTN3-rev: 5'-GGT CAC AGT ATG CAG GAG GG-3'. PCR reactions were set to a 25 µL final volume, with 10 mM Tris pH 8.4, 50 mM KCl, 1.75 mM MgCl₂, 0.1 % Triton X-100, 0.2 mM of each dNTP, 1 U Taq DNA polymerase, and 1.0 pM of each primer, using 100 ng of genomic DNA as template. The amplification program consisted of an initial denaturation step 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 step at 72 °C for 5 min. ACTN3 R577X alleles were distinguished by the presence (577 ×) or absence (577 R) of a restriction site of the enzyme DdeI [23]. For DdeI restriction digestion, 1 µL of PCR products was digested with 20 U of the enzyme in a final volume of 15 µL. Reactions were incubated overnight (O/N) at 37 °C. Restriction fragments were separated using electrophoresis in 8 % polyacrylamide gels and visualized with silver nitrate (O/N) at 37 °C. Restriction fragments were separated using electro-
Considering the characteristics of football, in which the outcome of the game is increasingly determined by strength and speed [38], and given the higher physical demands of training and games in senior categories, the lower frequency of the ACTN3-XX genotype in the professional category would be expected. Our results agree with previous findings on elite football players [34].

The higher frequency of the RX genotype, however, was indicative of the mixed character of this sport. It suggested that, in addition to strength and power, aerobic endurance is also important. Another relevant finding was the high frequency of ACTN3-RR individuals in the under-14 category. This was likely a result of timing; this is the stage at which athletes are selected for the first teams and power is still a critical factor, while over the years other characteristics also become crucial.

Other studies have found a relationship between the frequency of ACTN3-RR and the competitive level of some athletes [15, 17, 34, 41], which was not confirmed by other studies [10, 35]. What was remarkable about the present study was the higher frequency of ACTN3-RX in the senior categories; the categories more specialized for football.

The frequency of ACTN3 among 888 high-performing European athletes grouped into strength/power athletes, endurance athletes, and athletes taking part in collective disciplines of intermittent character, including football players, were compared [10]. The authors found higher frequencies of ACTN3-RR in strength/power athletes, in comparison to the collective and endurance disciplines groups. The authors argued that team sports not only have a mixed character, where both strength and endurance are important for good performance, but also depend on other abilities that interact with performance in team sports. The same conclusion has been obtained by other authors in a study on ACTN3 and team sport [20]. This corroborates the finding of the present study, in which the ACTN3-RX genotype was more frequently seen in senior football categories. These findings reinforce the need of both strength/power and endurance for good performance in football, as expected.

No allelic difference was found among athletes of high-level collective sports [35]; however, when a larger sample of athletes in individual sports was considered, higher levels of the R allele were found [41].

Football players in different divisions of the Spanish league were also studied [34]. The authors found that the RR and RX genotypes and the frequency of the R allele among football players were higher when compared to sedentary individuals and endurance athletes. According to the authors, the percentage of players with the XX genotype in the major league was lower. After comparing among different age categories, the present study obtained similar results. It has been observed that genetic profiles more suitable for strength or resistance present higher performance for specific training [16]. Probably this is a factor of longevity in the sport over the years in superior football categories.

Other studies have carried out cross-sectional examinations of the frequency of ACTN3-RR/RX in athletes from different disciplines with sometimes opposite results. A higher frequency of the ACTN3-RR/RX genotype was found in athletes from disciplines where strength and power are predominant [26, 28] and a higher frequency of ACTN3-XX was found in disciplines where aerobic endurance is predominant [41]. This relationship could not always be established [29, 35], which have been attributed to the small sample size in these studies or the low competitive level of the athletes taking part in them [29].

Studies of the relationship between sports performance and genetics can also be interpreted as an assessment of the genetic predisposition of certain populations [39]. In this study, there was no significant difference in genotype between the control group and any of the categories assessed. This fact should be interpreted with caution and does not justify the claim that the Brazilian population is predisposed to sports in which strength and power are predominant, or specifically for football, which can be classified as having a mixed (strength/power) character.

A relationship was found in the frequency of ACTN3-RR between Jamaican and American Olympic finalists in short-distance races and the populations of these countries [36]. Conversely, the same relationship between endurance athletes and African populations was not found [42], indicating that any relationship between the popu-
lation of certain countries and their genetic predisposition for a particular sport modality cannot yet be established with certainty.

Individually, it is still questionable if genetic profile analysis is important for directing future athletes towards disciplines for which they would show better aptitude, because talent is influenced by many other physical and personality characteristics. Even if genetic profile is a factor that interferes with high-performance sports success, other factors are also involved in this model [32].

For athletes whose genetic profile has been determined, the training program or recovery periods throughout a competition could be adapted [3, 16, 19, 43]. A higher susceptibility to muscle microtrauma in humans after eccentric exercise has been identified in ACTN3-XX individuals [18], including football players [21, 30] This ACTN3-XX propensity to muscle stress has not been confirmed by other studies [6], particularly those involving an animal model [5].

The homozygosity for the R577X (XX) allele results in a deficiency of α-actinin-3 protein in approximately ~18% of the healthy population of European origin, whereas other populations show different frequencies [23], such as in the case of Ethiopians (~11%) [41]. In the present study, ACTN3-XX presented a frequency of 14% in the control group, which did not differ from the groups of football players.

Nonetheless, it has not yet been conclusively demonstrated that the ACTN3-XX genotype is a possible selection factor for athletes. However, gene expression is only partially responsible for athletic success, and other biological characteristics as well as environmental issues cannot be overlooked [32, 35].

Conclusion

The distribution of the genotypic frequencies of the ACTN3 R577X polymorphism in football players did not differ compared to a control sample representative of the Brazilian population. There was a trend towards a higher RX genotype frequency and a lower XX genotype frequency in the professional category compared to the junior categories. These results suggest that the impact of ACTN3 XX genotype on the mixed characteristics (strength/endurance) that characterized the career of a football player also involves categories. These results suggest that the interaction between HIF1A P582S and ACTN3 R577X determinant for power/sprint performance? Metabolism 2010; 59: 861–865


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No conflict of interest has been declared by the authors.

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