**Significance of the Study**

- ACTN3 single nucleotide polymorphism allele distribution differs significantly according to a player’s field position.
- Identifying the genetic characteristics of a player to adapt his playing position may lead to a talent orientation in young football players.
- The ACTN3 gene may be considered as a potential biomarker for performance in football.

**Keywords**

Single nucleotide polymorphism · Football · Genetics · Performance

**Abstract**

**Introduction:** Football is characterised by intermittent high-intensity efforts varying according to the field position of a player. We aimed to ascertain whether polymorphisms in the ACTN3 gene are associated with different playing positions in elite professional football players. **Subjects and Methods:** Genotyping of the ACTN3 gene was conducted in 43 elite professional football players of a single team. Playing position was recorded based on the player’s most frequent position. **Results:** The genotype distribution was not significant between positions ($p = 0.057$), while the allele distribution differed significantly ($p = 0.035$). Goalkeepers ($p = 0.04, p = 0.03$), central defenders ($p = 0.03, p = 0.01$), and central midfielders ($p = 0.01, p = 0.00$) had a significantly different allele distribution compared with wide midfielders and forward players. **Conclusions:** Genetic biomarkers may be important when analysing performance capability in elite professional football. Identifying the genetic characteristics of a player to adapt his playing position may lead to orientation of positions based on physical capabilities and tissue quality in young football players, and also to performance enhancement in those who are already playing in professional teams.

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Introduction

The physiological demands of professional football call for the establishment of appropriate strategies for training, physical conditioning, and competition [1, 2] through objective quantification of the player’s workload and activities [3]. The application of Global Positioning System (GPS) satellite technology has allowed the collection and processing of large volumes of data more rapidly than hand-notation Time-Motion Analysis (TMA) did in the past [2].

In football, intermittent high-intensity efforts are performed within an endurance context [4, 5]. In a 90-min game, sprinting, jumping, kicking, turning, changing pace, and tackling take place with a high frequency [4]. TMA systems and concrete GPS technology have made it possible to characterise loads according to position on the field, offering teams the opportunity to individualise training aspects based on performance needs [6, 7]. Central defenders (CD) and central midfielders (CM) perform sprints and high-speed running to a lesser extent than do wide midfielders (WM), fullbacks (FB) and forward players (FW), whereas WM cover the largest distances [6, 7].

Performance capabilities are at least partially determined by genetics [8, 9]. Nucleotide variations or polymorphisms may influence phenotype, and single nucleotide polymorphisms (SNPs) account for 90% of these alterations [9]. Many genes with SNPs have been associated with performance in sport, in terms of endurance capabilities, muscle strength, or training response [8].

An SNP exists in the ACTN3 gene due to a cytosine (C) to thymine (T) transversion in position 1747 exon 16, converting an arginine (R) to a stop codon at residue 577 (R577X), thus causing 577X homozygotes to be completely deficient of the gene’s encoded protein, α-actinin 3 [10, 11]. The SNP of ACTN3 could be a potential biomarker of muscle performance, with allele 577R, more frequent in power athletes, favouring rapid and forceful muscle contraction [12–14].

The performance requirements of football players vary depending on their position on the field, and the SNP of ACTN3 is worth investigating as a potential biomarker of muscle performance. The aim of this study was to evaluate whether the genotype and allele distribution of the ACTN3 gene differs between positions on the field in elite professional football players.

Subjects and Methods

Study Population

The details of the study population have been previously described [15]. A total of 43 elite professional football players from a single club participated in the present study. The data collected included the descriptive statistics of demographic variables such as weight, height, ethnicity, age, the player’s position on the field, and the allele distribution of the ACTN3 gene’s polymorphism (R577X).

The position on the field was assigned according to the frequency that each player plays in each position, acknowledging that many of them are capable of playing in more than 1 position. Goalkeepers (GK), CD, FB, CM, WM, wingers (W), and FW were the categories for position on the field, as the participants belong to a team that plays a 4-3-3 formation.

DNA Extraction and Genotyping

The DNA extraction and the SNP analysis were conducted according to previous studies [15–18]. A real-time polymerase chain reaction (PCR) allelic discrimination TaqMan assay was performed. The procedure was undertaken according to the manufacturer’s (Applied Biosystems, Foster City, CA, USA) instructions with minor modifications.

Approximately 4 mL of whole blood was extracted from each participant into EDTA vacutainer tubes and stored at 4 °C for total DNA extraction. Genomic DNA isolation was undertaken using a QIAmp DNA Blood Minikit (Qiagen, Valencia, CA, USA), following the manufacturer’s instructions. DNA quantity was measured with a Nano-Drop ND-1000 Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). All the samples were stored at –80 °C until analysed.

Primers and probes were obtained from Applied Biosystems. A real-time PCR was performed on an ABI Prism 7500 Sequence Detection System (Applied Biosystems) following these conditions: 50 °C for 2 min, 95 °C for 10 min, and then 40 cycles of amplification (95 °C for 15 s and 62 °C for 1 min). For each cycle, the software measured the fluorescent signal from the VIC- or FAM-labelled probe. All PCRs were run in duplicate and contained 50 ng of DNA, 6.25 μL of TaqMan Universal Master Mix (Applied Biosystems), 0.25 μL of primers and probes, and water up to a final volume of 13 μL. Appropriate negative controls were run as well using water.

Statistical Analysis

The sample size was determined as the population in a specific professional football club, who were part of the club’s first team competing in the Spanish first division during the 2007–2012 and 2015–2017 seasons. Table 1 contains the descriptive statistics of demographic variables including age, ethnicity, weight, and height [15]. Genotype frequencies were calculated and compared with HapMap data (Table 2) [15].

Genotype and allele frequencies were calculated and compared with the field position of the players (Tables 3, 4) using a χ² test. In addition, allele frequencies were compared between each individual position on the field, once again using a χ² test for each of them (Table 5). All the statistical analyses were performed with SPSS version 21 for Mac. Significance was set at $p \leq 0.05$. 

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### Table 1. Characteristics of the study population

| Characteristic | Caucasian  
|               | (n = 23; 53.50%) | Black African  
|               | (n = 7; 16.30%) | Hispanic  
|               | (n = 13; 30.20%) | Total  
|               | (n = 43; 100%) |
| Age, years    | 28.04 (20–37) | 27.86 (21–34) | 27.46 (21–34) | 27.84 (20–37) |
| Weight, kg    | 74.87 (65–89) | 75.29 (69–90) | 74.6 (62–86) | 74.81 (62–90) |
| Height, cm    | 179.87 (170–194) | 179 (171–191) | 176.15 (169–186) | 178.61 (169–194) |

### Table 2. Genotype frequencies in the present study and for Caucasian (HapMap CEU), Black African (HapMap YRI) and Hispanic (HapMap HISP) populations in NCBI dbSNP

| Gene    | Genotype | Population total (n = 43), n (%) | Caucasian (n = 23), n (%) | HapMap CEU, % | Black African (n = 7), n (%) | HapMap YRI, % | Hispanic (n = 13), n (%) | HapMap HISP1, % |
|---------|----------|---------------------------------|--------------------------|--------------|-------------------------------|--------------|--------------------------|----------------|
| ACTN3   | RR       | 19 (44.19)                      | 9 (39.13)                | 19.46        | 5 (71.43)                     | 83.19        | 6 (46.15)                | N/A            |
|         | RX       | 21 (48.84)                      | 13 (56.2)                | 58.41        | 2 (28.57)                     | 16.81        | 5 (38.46)                | N/A            |
|         | XX       | 3 (6.97)                        | 1 (4.35)                 | 22.10        | 0 (0)                         | N/A          | 2 (15.39)                | N/A            |

NCBI, National Center for Biotechnology Information; dbSNP, Single Nucleotide Polymorphism database.

### Table 3. Genotype frequencies and position on the field comparison

| Gene    | Position          | Genotype distribution, n (%) | p value |
|---------|-------------------|------------------------------|---------|
|         | RR RX XX          |                              |         |
| ACTN3   | Goalkeeper        | 0 | 4 (100) | 0 |
| rs1815739 | Central defender | 2 (22.22) | 5 (55.56) | 2 (22.22) |
|         | Fullback          | 3 (42.86) | 4 (57.14) | 0 |
|         | Central midfielder| 0 | 2 (66.67) | 1 (33.33) | 0.053 |
|         | Wide midfielder   | 6 (75) | 2 (25) | 0 |
|         | Winger            | 3 (50) | 3 (50) | 0 |
|         | Forward player    | 5 (83.33) | 1 (16.67) | 0 |

### Table 4. Allele frequencies and comparison with field position

| Gene    | Position          | Allele distribution, n (%) | p value |
|---------|-------------------|----------------------------|---------|
|         | 577R 577X         |                            |         |
| ACTN3   | Goalkeeper        | 4 (50) 4 (50)              |         |
| rs1815739 | Central defender | 9 (50) 9 (50)              |         |
|         | Fullback          | 10 (71.43) 4 (28.57)       |         |
|         | Central midfielder| 2 (33.33) 4 (66.67) 0.035 |         |
|         | Wide midfielder   | 14 (87.5) 2 (12.5)         |         |
|         | Winger            | 9 (75) 3 (25)              |         |
|         | Forward player    | 11 (91.67) 1 (8.33)        |         |

### Table 5. Comparison of field position according to allele frequencies (p values)

| Position          | GK CD FB CM WM W FW |
|-------------------|---------------------|
| Goalkeeper (GK)   | – ns ns ns 0.04 ns 0.03 |
| Central defender (CD) | – – ns ns 0.03 ns 0.01 |
| Fullback (FB)     | – – ns ns ns ns ns ns |
| Central midfielder (CM) | – – – – 0.01 ns 0.00 |
| Wide midfielder (WM) | – – – – ns ns ns |
| Winger (W)        | – – – – – – ns |
| Forward player (FW) | – – – – – – |

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Results

Population

A total of 43 professional football players from a single club participated in the study, and their demographic variables can be seen in Table 1. Most participants were Caucasians (53.50%), followed by Hispanics (30.20%), and Black Africans (16.30%). The 43 players were divided into 4 GK, 9 CD, 3 CM, 8 WM, 6 W, and 6 FW.

Genotyping revealed that the 577R allele comprised 59 of the 86 alleles. A total of 19 individuals had the genotype RR, 21 had genotype RX, and 3 had genotype XX. The allele 577R was present in 93% of the participants. Genotype frequencies were compared with HapMap data (Table 2).

Genotype and Allele Distribution versus Position on the Field

Genotype RX was the most prevalent overall, being present in 48.83% (n = 21) of the subjects. Genotype RR was present in 44.19% (n = 19) of the participants, and 6.98% (n = 3) of the players had the XX genotype. In WM (75%), W (50%), and FW (83.33%) the genotype RR was the most frequent, and <50% presented the genotype RX (Table 3). Only CD and CM displayed the presence of genotype XX (22.22 and 33.33%, respectively), while GK and CM included no individuals with genotype RR. A comparison between genotype distribution and position on the field was close to being significantly different (p = 0.057).

Allele 577R was the most prevalent, accounting for 68.60%. Three positions on the field presented a distribution with a presence of 50% or less of allele 577R, namely GK (50%), CD (50%), and CM (33.33%). FW (91.67%), WM (87.5%), W (75%), and FB (71.43%), respectively, were the positions with the highest presence of allele 577R. Allele distribution differed significantly according to position on the field (p = 0.035).

When comparing each position on the field individually, GK had a different allele distribution than WM and FW (p = 0.04 and p = 0.03); CD (p = 0.03 and p = 0.01) and CM (p = 0.01 and p = 0.00) showed equal differences as well.

Discussion

To our knowledge, this is the first study to evaluate the role of genetics of elite professional football players according to their specific field position. The main finding in this study was that the ACTN3 SNP distribution was not significant (p = 0.057), and that the allele distribution differed significantly (p = 0.035) between the positions the players have on the field. More specifically, the allele distribution of GK, CD, and CM differed from that of WM and FW (p = 0.04, p = 0.03; p = 0.03, p = 0.01; and p = 0.01, p = 0.00; Table 5).

These results may be explained by the fact that the physical efforts required of the players during a match vary between different positions [6, 7, 19, 20]. The genetic makeup of professional football players is different from that of the normal population [21, 22], and is also different between professional and non-professional players [22].

The ACTN3 gene has been associated with physical performance [12–14, 23, 24]. The allele 577R is more frequent in individuals engaged in explosive activities [12–14, 23, 24], while allele 577X is over-represented in subjects engaged in endurance activities [16, 25]. In the elite professional football players involved in the present study, the heterozygous genotype RX is the most prevalent, in line with previous studies involving professional players [15, 22, 26, 27]. We are aware that other studies report the opposite, where the RR genotype was more prevalent than the RX genotype [21, 28, 29]. Nevertheless, not all of them involved solely elite professional football players [21].

Football is a sport performed in an endurance context in which intermittent high-intensity efforts occur [4, 5]. If we consider that the muscle performance of the ACTN3 gene varies between genotypes, RX individuals should generally adapt best to its physical effort requirements. RX is the most prevalent genotype among football players [15, 22, 26, 27]. On the other hand, the XX genotype is much less prevalent (nearly 7% of the subjects); theoretically, this genotype would fit less well with the physical requirements of football, as it is not conducive to explosive muscle performance [12–14, 23, 24]. Further, this study is in line with previous literature in which XX individuals are less prevalent among professional players compared with the normal population [15, 21, 22, 26, 28]. Finally, the RR genotype is present in 44.19% of individuals and is also the most prevalent in some studies [28, 29], as it is associated with the explosive muscle performance phenotype required in football. In this respect, therefore, genetics may be a factor for football players to become professional.

Physical efforts and conditioning needs differ, depending on the position players have on the field [6, 7, 19, 20], and, as seen in this and other studies, genotype and allele distribution may also vary [25]. In a football match, CM and CD run at high speed and sprint less than WM,
FB, and FW [6, 7], whereas CM jog more than FB and FW [6, 7]. Moreover, CD undertake the lowest high-intensity activities [19, 20], unlike WM and FW, who account for the highest peak game speed and frequency of high-intensity activities [19]. The allele distribution in this study shows that the explosive allele 577R is significantly less prevalent in CD (50%) and CM (33.33%) than in WM (87.5%) and FW (91.67%; p = 0.03, p = 0.01 and p = 0.01, p = 0.00; Table 4). Further, the XX genotype is only present in CD and CM (Table 3). These results are therefore consistent with the positional physical efforts described in the literature [6, 7, 19, 20]. Again, genetics may play a role for a player to reach professional level.

The RX genotype has been considered to be protective of soft-tissue musculoskeletal injury susceptibility in professional football [15], whereas genotype XX has been associated with a greater risk of injury [15]. The previous explanation of physical demands in football according to the field position and its association with genetic makeup agrees with the results of these injury studies, as genotype RX, which adapts most effectively to the demands in football, is the one with the lowest injury prevalence. On the other hand, genotype XX, which may be less suited to football, is associated with the highest odds of injury.

We are aware of the limitations of this study. First, all the players belong to a single club in which the 4-3-3 formation is used as the main tactic. Football matches differ in terms of demands and results. In addition, teams use different tactics and formations on the field based on a manager’s considerations and decisions. Moreover, physical training, the environment, season schedule, and ergonomic aids may vary between teams. It is not possible to consider all of these factors when associations between genotype and performance are undertaken. Finally, each player was classified according to the main position of play. However, as we pointed out, some players played in several positions, according to the tactical needs of the team. We are aware that this may have introduced a degree of uncertainty, but this is inevitable given the needs of a modern football team. As seen in Results, in some instances a playing position was not connected to a specific allele distribution.

Ideally, it would be interesting to be able to introduce as control a phenotype/genotype of a gene that is not thought to exert any influence on physical performance to make sure that there were no differences in this according to player position. This should be the basis for future endeavours in this field.

The results of the present study could provide clubs with a tool for talent orientation in young players, and guide performance enhancement training in professional players already performing at elite level. When these findings are combined with the fact that the ACTN3 gene has been shown to be a factor in soft-tissue musculoskeletal injuries incidence [15], it could also produce a reduction in injuries if players adapt their position on the field to match its genetic profiles. Thus, this study contributes to the endorsement of predictive genomics DNA profiling described in the past as a tool to be used by professional football clubs in order to establish a genetic-based targeted training [30]. Detecting abilities and weaknesses in association with sports performance can lead to individualised training programs, prevention protocols, and nutrition aids [30].

Conclusions

The recent literature on genetics in professional football suggests potential biomarkers of injury susceptibility and performance. This study suggests that the ACTN3 SNP gene may be a suitable biomarker for orientation of field position and performance enhancement, as physical capabilities and tissue quality vary among genotypes. Individualisation should be considered when assessing professional players and football teams in terms of performance enhancement and injury prevention.

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Statement of Ethics

This study was approved by the Ethics Committee of Universitat de Barcelona, Barcelona, Spain (Registry No. IBR00003099). All the football players were fully informed in writing about the procedures of the study, and they gave their signed informed consent to participate.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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