HUMAN MOVEMENT
2016, vol. 17 (4), 237–241

# VARIATION IN THE *ACE* GENE IN ELITE POLISH FOOTBALL PLAYERS

doi: 10.1515/humo-2016-0032

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ABSTRACT
**Purpose.** A common polymorphism in the angiotensin converting enzyme I gene (the *ACE* I/D variant) represents one of the first characterized and the most widely studied genetic variants in the context of elite athletes status and performance related traits. The aim of the study was to determine the genotype and allele distribution of the allele and genotype of the *ACE* gene in Polish male football players. **Methods.** The total of 106 Polish male professional football players were recruited. They were divided into groups according to the position in the field: forwards, defenders, midfielders, and goalkeepers. For controls, samples were prepared with 115 unrelated volunteers. DNA was extracted from the buccal cells donated by the subjects, and the PCR amplification of the polymorphic region of the *ACE* gene containing either the insertion (I) or deletion (D) fragment was performed. **Results.** The genotype distribution and allele frequencies among all football players did not differ significantly when compared with sedentary control individuals ($p = 0.887$, $p = 0.999$, respectively). Likewise, the analysis of forwards, defenders, midfielders, and goalkeepers revealed no significant differences in either *ACE* genotype or allele frequencies. **Conclusions.** We did not provide evidence for difference of variation of the *ACE* I/D polymorphism between Polish football players and controls, as we did not obtain any statistically significantly higher frequency of either of the analysed alleles (I and D) or genotypes (DD, ID, and II) in the studied subgroups. It may be suspected that harbouring of I/D allelic variants of the *ACE* gene neither decreases nor increases the probability of being a professional football player in Poland.

**Key words:** *ACE*, gene, football players

## Introduction

Football is the most popular sports game in the world, and requires a great versatility from the players. A football match places great aerobic and anaerobic burden on players [1–3] because of the number of high intensity actions involving sprints, jumps, one-to-one disputes, kicking the ball, and also because of the long distances the player is required to cover in 90 min [4]. A professional football player covers 8–12 km, out of which 10–20% corresponds to running bouts of near-maximal or maximal velocity performed in the determinant phases of a game [5, 6].

Human performance is affected by both the genetic makeup of an individual and the environmental factors [7]. The same applies to football players, whose performance is at least partly influenced by genetic components [8]. Even though it is difficult to determine the exact genetic fundaments of performance, there are many critical gene polymorphisms that have been reported to have a physiological impact on human performance. These gene variants have been called performance-enhancing polymorphisms and it was suggested that their simultaneous presence might affect the phenotype of high-level football players [9].

Among the several genes related to sports performance [10], the gene encoding the angiotensin-converting enzyme (*ACE*) is one of the most intensively investigated [11]. The human angiotensin converting enzyme gene (*ACE*) is located on chromosome 17 in position 17q23.3 [12]. The product of this gene (an enzyme converting angiotensin I into II) is acknowledged to be a key element in the renin-angiotensin system (RAS), responsible for the regulation of blood pressure – one of the main factors determining the efficiency of the whole body. The

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most widely studied *ACE* polymorphism is the 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. In this case, three *ACE* genotypes include DD (deletion/deletion) and II (insertion/insertion) homozygotes and ID heterozygotes (insertion/deletion). The I allele is associated with lower ACE activity in both serum and tissue as compared with the D allele [13].

Many of the case-control studies have reported that success in speed-strength disciplines, such as short-distance running, long jump, high jump, and short-distance swimming, is associated with *ACE* DD genotype [14–16]. In turn, individuals with the *ACE* II genotype have more success in endurance-related disciplines, such as medium- and long-distance running, race walking, hockey, and rowing [17–19]. Nevertheless, some studies do not confirm these observations [20].

Not clearly enough, is also the role of the *ACE* I/D polymorphism in football. There are few articles discussing the problem. The first study analysed the difference in *ACE* alleles and genotype distribution between football players and showed higher proportion of the DD genotype of the I/D polymorphism in the *ACE* gene in world-class professional soccer players compared with non-athletic controls and elite endurance athletes [21]. On the other hand, other studies, involving Lithuanian football players, proved that players had a significantly higher percentage of the ID of *ACE* genotype (but not the DD genotype) as compared with the nonathletic population [22]. These observations indicate that the status of the *ACE* gene I/D polymorphism is still unclear. What is more, the distribution of the *ACE* gene has not ever been investigated with reference to Polish football players.

The aim of this study was to determine the genotype and allele frequency distribution of the *ACE* gene I/D polymorphism in Polish male football players.

## Material and methods

### Ethics Committee

The Pomeranian Medical University Ethics Committee approved the study, and written informed consent was obtained from each participant. The study complied with the guidelines set out in the Declaration of Helsinki.

### Subjects

The samples for investigation were collected in year 2009 (since that moment, the isolates have been used in several studies, for example concerning the genes coding collagen proteins; the *ACE* gene is the next step previously planned in the experiment). All participants recruited to the study ($n$ = 106) were Polish male football players from the best Polish football teams (Piast Gliwice, Podbeskidzie Bielsko-Biała, Wisła Kraków, Cracovia Kraków, Polonia Bytom, and Odra Wodzisław). For the analysis, the football players were divided into groups according to the position in the field: forwards ($n$ = 28), defenders ($n$ = 38), midfielders ($n$ = 32), and goalkeepers ($n$ = 8). The control group consisted of healthy individuals ($n$ = 115, all males), also selected from the Polish population (sedentary college students). The geographic origin of the football players and control group participants was self-reported and all the studied individuals were Caucasians.

### Genotyping

The buccal cells donated by the subjects were collected in the Resuspension Solution (Sigma, Germany) with the use of Sterile Foam Tipped Applicators (Puritan, USA). DNA was extracted from the buccal cells with the GenElute Mammalian Genomic DNA Miniprep Kit (Sigma, Germany) according to the producer's protocol.

### *ACE* genotyping

PCR amplification of the polymorphic region of the *ACE* gene containing either the insertion (I) or deletion (D) fragment was performed. Only one pair of primers (*ACE* forward: CTG GAG ACC ACT CCC ATC CTT TCT and *ACE* reverse: GAT GTG GCC ATC ACA TTC GTC AGA) was used to determine the *ACE* genotype, yielding amplification products of approximately 490 bp (for the I allele) and 190 bp (for the D allele). The 10 µl PCR consisted of: 1 µl DNA isolate; 0.5 U DNA recombinant Taq polymerase in buffer (pH = 8.0; Sigma, Germany); 1x PCR buffer (pH = 8.7; Sigma, Germany); 1.5 mM $MgCl_2$; 4 pM primers (Oligo, Poland) in TE buffer (pH = 8); 0.75 nM of each dNTP. The thermal-time PCR was as follows: initial denaturation at 94°C for 300 s, 30 cycles (denaturation at 92°C for 60 s, primer annealing at 58°C for 60 s, chain extension at 72°C for 150 s), and final extension at 72°C for 360 s. The reaction was performed in two samples per isolate. Amplification products were visualized in UV light with the use of 1.5% agarose gels stained with ethidium bromide.

### Statistical analyses

Allele frequencies were determined by gene counting. Genotype distribution and allele frequencies were compared between groups of athletes and controls and significance was assessed by $\chi^2$ analysis for the biallelic polymorphism in the *ACE* gene. The $p$ values of $< 0.05$ were considered statistically significant.

## Results

The *ACE* I/D genotype frequencies among all football players, players according to their position in the

field, as well as in the control group conformed to Hardy-Weinberg expectations (Table 1). The genotype distribution and allele frequencies among all football players did not differ significantly when compared with sedentary control individuals ($p = 0.887$, $p = 0.999$, respectively, Table 1). Likewise, the analysis of forwards, defenders, midfielders, and goalkeepers revealed no significant differences in either *ACE* genotype or allele frequencies.

## Discussion

In the present study, we have investigated variations of the *ACE* I/D polymorphism in Polish elite football players. Our main finding was the lack of any statistically significant differences concerning the allele and genotype distribution between football players and controls.

In spite of the lack of statistical significance, the study has several strengths. To the best of our knowledge, this is the first study in the scientific literature examining the relationship between the *ACE* I/D polymorphism in Polish football players. It is worth to point that although the effect of the *ACE* I/D polymorphism on exercise performance has been the subject of much research, most studies do not refer to team games.

The second important finding of the study is the estimation of the *ACE* I/D polymorphism as a genetic marker used in Talent Search type projects. The *ACE* gene is not only the most intensively investigated gene in sports sciences, but also the most frequent component (61% of all tests) of genetic tests proposed by the direct-to-consumer genetic testing companies [23]. The lack of statistical significance of our results permits to verify the usefulness of this kind of tests prepared on the basis of Polish best athletes and proposed in Poland.

As mentioned above, there have been some previous studies conducted on the evaluation of the *ACE* ID genotype among football players, but none of them has been performed among the Polish population. What is more, generally our findings are opposite to most previous studies. Gineviciene et al. [21] analysed 199 Lithuanian professional soccer players and observed higher frequencies of the *ACE* ID genotype in the player groups versus the control groups. Juffer et al. [22] showed that among 54 professional soccer players, the frequency of the heterozygous *ACE* ID genotype was higher, and the frequency of the *ACE* II genotype was lower when the players were compared with long-distance runners. Similar results were obtained by Ulucan et al. [7], who investigated 25 professional football players. They also found the overrepresented DD and ID genotype in the studied athletes ($n$ = 88 DD + ID genotype vs. 12% II genotype). The findings of Ulucan et al. [7] were supported by the work by Egorova et al. [24], who examined 213 Russian football players and observed the frequency of ID and DD genotypes equal 28.6% and 50.7%, respectively. This gave both genotypes an overall frequency of 79.3%, which is similar to our results.

Our results were in opposition to the investigations listed above. We did not found any statistically significant differences of the *ACE* I/D allele or genotype distribution between the football players and controls, but also among the football players divided into groups according to the position in the field: forwards, defenders, midfielders, and goalkeepers. What is worth noticing, our results are similar to those obtained by Massidda et al. [25], who also did not identify differences in the genotypic distribution of the *ACE* I/D in a group of 30 Italian soccer players and a sample from the general population. In addition, because both the control group and the athletes presented a high *ACE* DD frequency (45% and 60%, respectively), the authors reported that this gene was not responsible for the success of players in football.

The differences concerning our findings and the listed above investigations by Gineviciene et al. [21],

Table 1. The *ACE* I/D polymorphism allele and genotype frequencies for the footballers and the control group

| Groups | | Forwards | Defenders | Midfielders | Goalkeepers | All | Controls |
|---|---|---|---|---|---|---|---|
| Size, *n* | | 28 | 38 | 32 | 8 | 106 | 115 |
| Allele (%) | I | 24 (42.8%) | 32 (42.1%) | 31 (48.4%) | 7 (43.7%) | 94 (44.4%) | 102 (44.3%) |
| | D | 32 (57.2%) | 44 (57.9%) | 33 (51.6%) | 9 (56.3%) | 118 (55.6%) | 128 (55.7%) |
| *p* | Compared with control | 0.840 | 0.733 | 0.561 | 1.0 | 0.999 | – |
| Genotype (%) | II | 5 (17.8%) | 7 (18.4%) | 8 (25.0%) | 2 (25.0%) | 22 (20.8%) | 22 (19.1%) |
| | ID | 14 (50.0%) | 18 (47.4%) | 15 (46.9%) | 3 (37.5%) | 50 (47.2%) | 58 (50.4%) |
| | DD | 9 (32.2%) | 13 (34.2%) | 9 (28.1%) | 3 (37.5%) | 34 (32.0%) | 35 (30.5%) |
| *p* | HWE | 0.920 | 0.862 | 0.729 | 0.502 | 0.647 | 0.823 |
| | Compared with control | 0.979 | 0.909 | 0.767 | 0.777 | 0.887 | – |

HWE – Hardy-Weinberg expectation

Juffer et al. [22], Ulucan et al. [7], or Egorova et al. [24] can be explained by a variation among populations of different geographic and ethnic backgrounds. In this sense, we cannot exclude the possibility that our results are unique to the specific Polish population of elite football players examined in our study.

Generally, genetic investigations in sport are most representative in disciplines with endurance or power playing a key role [26, 27]. The lack of association between genetic markers and team-sports performance could be presumably due to the mixed nature of team-sports events, in which both the aerobic and anaerobic energy systems are important factors of a successful performance. What is more, the tactics and technique are important at the same level as physical performance, probably remaining impossible to explain by genetic factors.

In conclusion, we did not provide evidence for a difference in variation of the *ACE* I/D polymorphism between Polish football players and controls, as we did not obtain any statistically significantly higher frequency of either of the analysed alleles (I and D) or genotypes (DD, ID, and II) in the studied subgroups. It may be suspected that harbouring of I/D allelic variants of the *ACE* gene neither decreases nor increases the probability of being a professional football player in Poland. Nevertheless, our study should be taken into consideration with caution when referring to the main limiting factor in the field of sports genetics, i.e. samples of investigated athletes large enough to obtain valid results.

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Paper received by the Editor: October 14, 2016
Paper accepted for publication: December 5, 2016

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