

## *ACE* I/D genotype in professional field hockey players

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**Introduction.** Numerous studies have focused on the association between I/D *ACE* and physical fitness; however, this association in professional hockey players has never been recognized. **Aim of the Study.** The study examined the distribution of *Alu* insertion (I)/deletion (D) angiotensin converting enzyme (*ACE*) genotypes in elite male professional field hockey players. **Material and Methods.** The effect of *Alu* insertion (I)/deletion (D) angiotensin converting enzyme (*ACE*) genotypes on motor skills and maximal oxygen uptake ( $VO_2\max$ ) in 47 elite male field hockey players was studied. Genotyping for *ACE* I/D was performed using a polymerase chain reaction on DNA from leucocytes. The studied motor skills such as speed – 20 m run, power – vertical jump, recovery – step-test, speed endurance (15 x 20 m) shuttle run, were established using functional tests. The  $VO_2\max$  was measured during progressive exercise test till exhaustion. **Results.** The authors do not confirm some literature data that D allele favours endurance ability. We did not detect significant genotype effects of *ACE* on the analyzed traits; however a tendency for decreased performance of individuals with the DD genotype was noted for vertical jump, power peak and power. **Conclusions.** Analysis of the genetic profile of *ACE* I/D may provide supplementary information on a player's predispositions to exercise with specific energy requirements.

**KEY WORDS:** *ACE* gene, athletic performance, genetic polymorphism, field hockey.

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### What is already known on this topic?

The *ACE* gene might be the “speed/endurance gene”. *Alu* Insertion polymorphism is associated with endurance (cycling, long distance running), unlike Deletion polymorphism which is associated with sprint/power events (sprints, velodrome cycling, short distance swimming, etc.). There are no data available on field hockey players.

### Introduction

Exercise performance is a multifactorial, quantitative trait resulting from an interaction of genetic and environmental compounds. Genetic factors have an important contribution to the variance between individuals. However there are no literature data concerning the genetic background of physical performance in field hockey but only few genotype frequency investigations in team games [1].

The prevalence of aerobic energy usage in field hockey draws the attention of researchers also to genes associated with endurance performance. Several studies have

suggested that the human angiotensin-converting enzyme (*ACE*) gene polymorphism is one of them. Although the genetic variants in *ACE* are not associated with a known disease phenotype, it is believed to preclude an *Alu* insertion (I) variant rather than the deletion (D) variant of the human angiotensin-converting enzyme (*ACE*) gene seemed to be associated with elite endurance performance [2]. However, some authors [3, 4, 5] do not confirm significant associations between I/D polymorphism in *ACE* gene and maximal oxygen uptake ( $VO_{2max}$ ). Amir et al. [6] report directly that their main finding is the overrepresentation of the *ACE* D allele and the DD genotype among elite Israeli marathon athletes so it favours improved endurance ability. The aforementioned facts – lack of literature data on genetic determination of field hockey players performance and ambiguity of the association of I/D alleles in *ACE* gene with aerobic or anaerobic energy usage inspired us to perform a study on genetic effects on performance of field hockey players.

It should be stressed that *ACE* is a crucial component of the renin–angiotensin–aldosterone system (RAAS) which sustains the circulatory homeostasis also (or especially) during its disturbances, including physical exercise. During physical exercise renin, secreted by the kidneys into the bloodstream, catalyzes angiotensin I, which after its conversion into angiotensin II serves as a vasoconstrictor and, in effect, increases blood pressure [7] and activates aldosterone – a regulator of water-mineral balance. The *ACE* gene is widely expressed in human tissues, including skeletal muscles [8]. The frequency of genotypes II and ID is higher in athletes practicing sports requiring a high aerobic capacity, e.g. rowers [5, 9]. Genotype II features a low *ACE* activity, which permits maintaining good balance without high blood pressure. On the other hand, a high level of *ACE* is more frequent in athletes practicing sports involving anaerobic metabolism: sprinters, short-distance swimmers. The level of motor abilities of field hockey players, like football players, depends on their playing position. Aerobic endurance is an ability required from defensive hockey players; while speed endurance is required from strikers. The purpose of our investigation was to study the frequency of distribution of an *Alu* insertion (I)/deletion (D) angiotensin converting enzyme (*ACE*) genotypes in elite male professional field hockey players.

#### Aim of Study

The present study was carried out on professional field hockey players. The following research question was for-

mulated: Is there any association between the I/D *ACE* genotype and physical performance tests and maximal oxygen uptake in professional field hockey players?

#### Methods

##### Subjects

The study protocol was approved by the Bioethical Committee of the Poznań University of Medical Sciences (no. 1060/05) and was in accordance with the Declaration of Helsinki for Human Research. Each of 47 male field hockey players signed a consent form. The players were 18-33 years old All participants were of Caucasian origin (100%). The sample consisted of 13 strikers, 16 midfielders and 18 defenders. Due to the specificity of play in particular tactical formations, the goalkeepers were excluded. The players' average age was  $24 \pm 5.7$  (mean  $\pm$  SD) years. 9 players took part in the Sydney Olympics, 16 won the second place in the Indoor Field Hockey World Cup, 20 were members of the Polish Senior National Team, 16 were from the Junior National Team and the remaining players were members of the champion and vice-champion teams of the Polish field hockey league.

##### Genotyping

Genetic analyses were conducted at the Laboratory of Genetic Analyses at the University School of Physical Education in Poznań, certified by ISO 9001:2008 standards (no. 69178-2009-AQ-POL-RvA). Genomic DNA was extracted from peripheral blood collected from the participants onto anticoagulant (EDTA). DNA isolation was performed using guanidine isothiocyanate (GTC, Sigma) method. An *Alu* insertion (I)/deletion (D) angiotensin converting enzyme (*ACE*) polymorphism was genotyped by polymerase chain reaction (PCR). DNA was amplified in a volume of 20  $\mu$ l. Genomic DNA from each examined individual was analyzed in 4  $\mu$ l (200 ng) and 16  $\mu$ l reaction mixture, containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM  $MgCl_2$ , 0.25 mM dNTP, 7.5 pmol each primer and 0.5 unit of Taq polymerase (Fermentas Life Sciences, Lithuania). The primers sequence was: F- CTg gAg ACC ACT CCC ATC CTT TCT and R- gAT gTg gCC ATC ACA TTC gTC AgA T [10]. The 35 cycle reaction was run in a Biometra T-personal thermocycler. The cycle comprised initial denaturation at 95°C for 10 min, denaturation at 95°C – 30 s, annealing at 55°C – 30 s, synthesis at 72°C – 30 s and final synthesis at 72°C for 10 min. PCR products were separated on 2% agarose gel. Electrophoresis

was run at 100 V for 30 min in Biometra agagel mini horizontal apparatus (Germany) and the results were visualized on a UV transilluminator with ethidium bromide (5 mg/ml).

#### Energetic features and functional capacities

Four functional tests were administered in the following order: maximal running speed (20 m sprint), power of lower limbs (vertical jump, VJ), speed–endurance (repeated sprint shuttle run). The rest interval between the tests was 10 minutes.

**Maximal speed of running (anaerobic effort).** The athlete ran a distance of 20 m from a standing start 0.5 m behind the starting line. The time from crossing the starting line to crossing the end line was measured to the nearest 0.001 s using a digital laser photocell system [11]. The run was repeated twice. The better time was converted to velocity [ $\text{m} \cdot \text{s}^{-1}$ ] for the analysis.

**Power of lower limbs (anaerobic effort).** The athlete stood sideways to the wall bars with a measuring board and reached up with the hand closest to the highest but natural point. Keeping the feet flat on the ground, the point reached by the fingertips was marked and recorded. It was called the standing reach height. The athlete then stood away from the wall, and leaped vertically as high as possible using both arms and legs to assist in projecting the body upwards, attempting to touch the wall at the highest point of the jump. The difference in distance between the standing reach height and the jump height was the score. Three attempts were recorded but for the analysis the best result was used. The result in

centimetres (cm) was converted to peak (PP) and average power (PA) in watts [W], using the Johnson and Bahamonde formula [12]:

$$\text{Power-peak [W]} = 78.6 \cdot \text{VJ (cm)} + 60.3 \cdot \text{mass (kg)} - 15.3 \cdot \text{height (cm)} - 1,308$$

$$\text{Power-avg [W]} = 43.8 \cdot \text{VJ (cm)} + 32.7 \cdot \text{mass (kg)} - 16.8 \cdot \text{height (cm)} + 431$$

**Speed endurance** (combination of running speed and aerobic endurance or anaerobic–aerobic capacity) was measured with a repeated 20 m shuttle run protocol. After a 10 minute rest prior to the test, the subject ran a series of 15, 20 m sprints with a 30 s interval between sprints (during which the subject walked back to the starting line). Time was measured to the nearest 0.001 s using a digital laser photocell system. The times to cover each sprint and the rest intervals were summed and converted to a velocity [ $\text{m} \cdot \text{s}^{-1}$ ]. The details, accuracy and diagnostic value of the test have been described in literature [11].

#### Maximal oxygen uptake

Physiological analyses were conducted at the Laboratory of Functional Examinations at the University School of Physical Education in Poznań, certified by ISO 9001:2008 standards (no. 69178-2009-AQ-POL-RvA). In order to determine the maximal oxygen uptake the direct method during exercise tests on a treadmill (Woodway, USA) was used. During each test, the composition of  $\text{VO}_2$  was analysed by the Oxycon Mobile spirometer (Jaeger, Germany). The exercise tests were carried out on a treadmill with increasing load, starting from a running speed of 8 km/h, increasing the load by 2 km/h

**Table 1.** Performance results of Elite Polish field hockey athletes classified by I/D allele of *ACE* gene

| Variable   | ACE                   |        |                       |        |                       |        | p     |
|--|-----------------------|--------|-----------------------|--------|-----------------------|--------|-------|
|  | DD<br>(n=13)<br>(28%) |        | ID<br>(n=20)<br>(42%) |        | II<br>(n=14)<br>(30%) |        |       |
|  | M                     | SD     | M                     | SD     | M                     | SD     |       |
| Vertical jump [cm]   | 51.4                  | 4.77   | 54.1                  | 5.89   | 54.5                  | 7.41   | 0.303 |
| Peak Power [W]   | 5661.95               | 689.74 | 5980.23               | 648.35 | 5914.22               | 720.58 | 0.647 |
| Power Average [W]  | 2069.49               | 353.56 | 2220.86               | 324.06 | 2192.05               | 404.00 | 0.763 |
| Sprint [ $\text{m} \cdot \text{s}^{-1}$ ]  | 6.541                 | 0.16   | 6.604                 | 0.19   | 6.620                 | 0.22   | 0.365 |
| Speed-endurance [ $\text{m} \cdot \text{s}^{-1}$ ]                                 | 6.342                 | 0.17   | 6.340                 | 0.21   | 6.384                 | 0.24   | 0.365 |
| $\text{VO}_2\text{max}$ [ $\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ] | 54.16                 | 4.74   | 52.89                 | 4.76   | 55.04                 | 4.05   | 0.242 |

every 3 min, until the moment of maximum individual load was reached.

### Statistical analysis

The effect of genotype on fitness was evaluated using analysis of covariance in PROC GLM of the SAS package (SAS Institute Inc. 2002-2003. The SAS System for Windows version 9.1. Cary, NC 27513-2414 USA) fitting age effect as a covariate.

### Results

The analysis of covariance did not detect significant genotype effects of *ACE* on the analyzed traits, however a tendency for decreased performance of individuals with the DD genotype was noticeable for vertical jump (LSMeans for DD = 51.04, DI = 54.30, II = 54.53), power peak (LS Means for DD = 5570, DI = 6035, II = 5921) and power average (LS Means for DD = 2021, DI = 2250, II = 2196) (Table 1). The contrast between DD and DI and II individuals suggested some possibility of association with VJ (p value 0.120); PP (p value 0.085); PA (p value 0.104). For recovery index (LS Means for DD = 106.5, DI = 97.0, II = 107.5) and maximal oxygen uptake (LS Means for DD = 54.9, DI = 52.5, II = 55.0) heterozygous individuals tended to perform slightly worse than the two homozygotes. The contrast of heterozygote vs the homozygotes showed suggestive p values of 0.083 for RI and 0.081 for  $\text{VO}_{2\text{max}}$ .

#### What this study adds?

This is the first study that has examined the effect of an athlete's *ACE* genotype on actual field hockey players' motor skills and aerobic endurance. We did not detect significant genotype effects of *ACE* on the analyzed traits; however, a tendency for decreased performance of individuals with the DD genotype was noted for vertical jump, power peak and power average.

### Discussion

This is the first report on the frequency distribution of an *Alu* insertion (*I*)/deletion (*D*) angiotensin converting enzyme (*ACE*) genotypes for motor skills and maximal oxygen uptake in elite field hockey players. It is of interest because (1) field hockey is a sport of light and moderate intensity of intermittent nature (aerobic energy – 60% of playing time, anaerobic energy – 40%). The remaining parts of a 70-min field hockey match

are intensive submaximal and supramaximal activities [13, 14]. The average distance covered by a field hockey player during a game amounts to 6,500-9,500 meters [15, 16]. During a game a hockey player performs 780-900 motor activities [14, 15], including 30-36, 2-6-sec sprinting activities [13, 14, 15]. The intensity of a hockey player's efforts is magnified by the characteristic semi-crouched posture while moving with and without the ball [17]. Thus a field hockey game involves a great energy expenditure of about 10-12 kcal/min [18, 19] and mean heart rate of 150-170 b/min [13, 14, 20, 21].

Each quantitative trait of a phenotype is a result of genetic and environmental factors and interactions between them. For coaches, data on players' predispositions will be significant in the future for selection or playing position in field hockey. The tendencies noted in the present study can be a significant contribution to the discussion.

### Conclusions

Recent years have witnessed an intensive development of research into the establishment of athletes' genetic profiles in order to determine their predispositions towards specific physical efforts or motor traits. Analysis of the genetic profile of *ACE* I/D may provide supplementary information to coaches on field hockey players' predispositions.

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