THE ANGIOTENSIN CONVERTING ENZYME GENE I/D POLYMORPHISM IN ELLITE POLISH AND LITHUANIAN JUDO PLAYERS

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ABSTRACT: 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 this study is to perform preliminary studies to analyze the possible importance of the ACE gene polymorphisms in elite Polish and Lithuanian judo players and sedentary individuals representing the possible relationships with genotype and physical performance. 28 male of elite Polish and Lithuania judo players were recruited for this study. For controls samples were prepared from 115 unrelated volunteers. DNA was extracted from the buccal cells donated by the subjects, and the PCR amplification of the polymorphic region of ACE gene contained either the insertion (I) or deletion (D) fragment was performed. Compared with sedentary controls, the frequency of I allele differ significantly from that found in judo player's group: 60.7% vs. 44.3%, ($p=0.02$) and ACE genotype frequency amongst the whole athletes group (28.6% II, 64.3% ID, 7.1% DD) was also different from expected values (control group 19.1% II, 50.4% ID, 30.4% DD; $p=0.019$). Our investigation have proved the ACE I/D allele could be one of the factors influencing the elite endurance exercise performance. The research suggests that those most predisposed to judo are individuals with the allele distribution in the ACE gene that is most significant with regard to the duration of a fight, and not with regard to the character of the performed moves, as could be supposed.

KEY WORDS: ACE, gene polymorphism, judo

INTRODUCTION

ACE is the most often investigated gene in the context of genetic conditioning of sports-predispositions. 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 [7].

The angiotensin converting enzyme (products of ACE expression) is a zinc membrane-bound metallopeptidase that governs the conversion of angiotensin I to angiotensin II and the degradation of bradykinin at the endothelial surface, thus being important in vasoactive peptide metabolism [18]. The regulation and control of ACE gene expression is still largely unknown and might be tissue-specific [2].

The human ACE gene is found on Chromosome 17 in position 17q23.3 with 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 [16,17].

Three ACE genotypes include D/D and I/I homozygotes and I/D heterozygotes [24]. The I allele is associated with lower ACE activity in both serum and tissue compared with D allele [15].

Allele D has been shown in association the increase in concentration of ACE enzyme in blood and tissues, which is conducive to an increase in blood pressure, a predisposition to hypertension and the hypertrophy of the left heart ventricle [3,20,21].

Allele I has been shown in association the increase in the endurance of muscles, their effectiveness and is also responsible for an increase in the proportion of free fibers (type I muscle fibers) [7, 25].

The features and known biological functions of ACE have been summarized for example in a review written by Coates [4].

The aim of this study is to perform preliminary studies to analyze the possible importance of the ACE gene polymorphisms in elite Polish and Lithuanian judo players and sedentary individuals representing the possible relationships with genotype and physical performance.

TABLE I. FREQUENCY OF THE ACE INSERTION (I) AND DELETION (D) ALLELES AND GENOTYPES. NUMBERS ARE ABSOLUTE VALUES AND PERCENTAGE FREQUENCIES ARE IN PARENTHESES, N IS A NUMBER OF SUBJECTS STUDIED.

Group	ACE allele		ACE genotype		
	I	D	II	ID	DD
Elite judo players (n=28)	34 (60,7%)	22 (39,3%)	8 (28,6%)	18 (64,3%)	2 (7,1%)
Controls (n=115)	102 (44,3%)	128 (55,7%)	22 (19,2%)	58 (50,4%)	35 (30,4%)

MATERIALS AND METHODS

Subjects: 28 male of elite Polish and Lithuania judo players were recruited for this study. 26 of them were national representatives with no less than ten years experience participating in sport. All of them were the Poland or Lithuania National Championship medalist. Additionally 5 of them were European Championship medalist, 4 of them were World Championship medalist and 2 of them were Olympic Game medalist.

For controls, samples were prepared from 115 unrelated volunteers (male students of University of Szczecin, aged 19-23). The athletes and controls were all Caucasian to ensure no likely racial gene skew and to overcome any potential problems of population stratification. The procedures followed in the study were approved by the Pomeranian Medical University Ethics Committee.

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

PCR amplification of the polymorphic region of ACE gene contained either the insertion (I) or deletion (D) fragment was performed. Only one pair of primers (ACEfor: CTG GAG ACC ACT CCC ATC CTT TCT and ACErev: GAT GTG GCC ATC ACA TTC GTC AGA) was used to determine the ACE genotype, yielding amplification products of approximately 490 bp (for I allele) and 190 bp (for D allele), as it have been reported elsewhere [21]. 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); 15 mM MgCl2; 4 pM primer ACEfor and ACErev (Oligo, Poland) in TE buffer (pH=8); 0,75 nM of each dNTP. Amplification was performed in a Peltier Thermal Cycler PTC-200 MJ Research (USA) and a T-Gradient Biometra (Germany). The thermal-time PCR was as follows: initial denaturation at 94oC for 300 s, 30 cycles (denaturation at 92oC for 60 s, primer annealing at 58oC for 60 s, chain extension at 72oC for 150 s) and final extension at 72oC for 360 s. The reaction was performed in two samples per isolate. Amplification products were visualized by using 1.5% agarose gels stained with ethidium bromide.

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

RESULTS

Amplification with primers ACEfor and ACErev in two samples gave a product of 190 bp representing a deletion fragment of the ACE gene. In 8 samples there were products of 490 bp characteristic for insertion allele of the ACE gene, whereas in 18 samples both products were observed (Fig. 1).

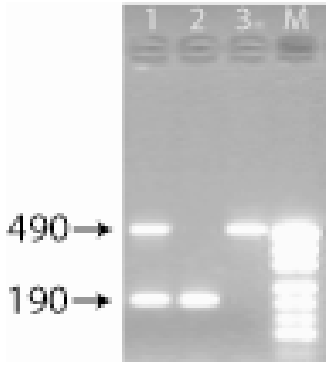

FIG. 1. EXEMPLARY AMPLIFICATION PRODUCTS OF ACE GENE WITH ACEFOR AND ACEREV PRIMERS. LANES: 1 – ID HETEROZYGOTE, 2 – DD HOMOZYGOTE, 3 – II HOMOZYGOTE, M – MOLECULAR WEIGHT MARKER.

ACE genotype distributions amongst subjects and controls were in Hardy-Weinberg equilibrium, making selection bias less likely. The distributions of the ACE genotypes and alleles are given in Table 1.

The genotype distribution (Fig. 2) amongst the whole cohort of athletes (28.6% II, 64.3% ID, 7.1% DD) was significantly different to that amongst sedentary controls (19.1% II, 50.4% ID, 30.4% DD; p=0.019); a significant excess of the I allele (Fig. 3) being noted in the whole cohort of the judo players (60.7% vs. 44.3%, p=0.02).

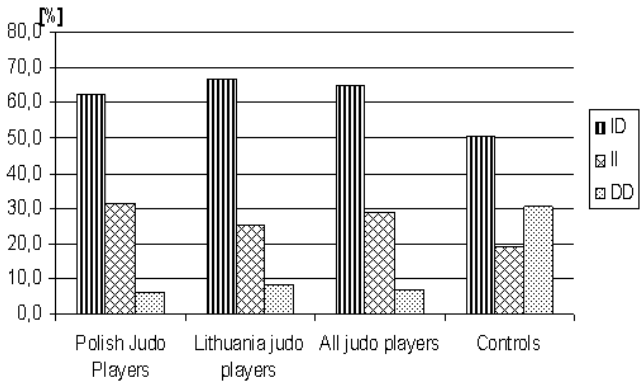

FIG. 2. ACE GENOTYPE FREQUENCIES IN 28 POLISH AND LITHUANIAN JUDO PLAYERS AND CONTROLS.

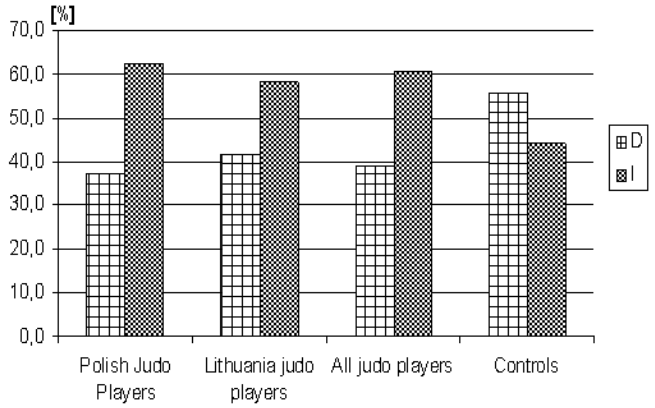

FIG. 3. I AND D ALLELE FREQUENCIES IN 28 POLISH AND LITHUANIAN JUDO PLAYERS AND CONTROLS.

DISCUSSION

Sport performance is a multifactorial phenotype, influenced by several factors, such as physique, biomechanical, and social characteristics [14]. It is considered that phenotypes variability in greatest range depends on DNA sequence polymorphism.

First reports on the ACE gene I/D polymorphism have concerned the health implications [3,20]. Till this moment the role of ACE gene for health have been intensively explored in more then 140 separate studies.

I/D polymorphism in intron 16 of the gene coding for the ACE has been also used to study the role of this gene in the physical performance. Mostly the case-control studies have shown a significant association between ACE genotype and elite athlete status [7]. The first such study reported an increased frequency of the I allele in British high-altitude mountaineers compared controls [9]. Many further studies have shown that not only I allele, but two alleles at ACE I/D polymorphism have differing effects on athletic performance [11,23].

It was suggested that the I allele of the ACE gene may be associated with improved performance and this effect might influence general sport ability. Investigations have shown that ACE I allele is associated with improved muscle efficiency [22], greater anabolic activity in response to physical training [10], explained by an increased intake of oxygen by the muscular tissue [7]. Allele I is also responsible for an increase in the proportion of free fibers (type I muscle fibers) in the lateral vastus thigh muscle in non-sporting individuals [25].

The D allele is described to be a factor responsible for the increased power of a sprinter and the power of muscles, confirmed by a research on short-distance swimmers [23], which showed a greater frequency of allele D than the non-training control group). One of the probable causes of this phenomenon could be a hypertrophy of the muscular tissue. Analyses show that allele D is related to an increase in the strength of the quadricipital thigh muscle in a response to a nine-week isometric-strength training [5]. These facts may be a proof the ACE D allele has been associated with higher ACE activity, compared to the ACE I allele [1,6]. The study have supported the interpretation concerned ACE activity is Montgomery research [8], where individuals with D/D genotype shown a significantly increased left ventricular mass in responsible to physical training, compared to the I/I and the I/D genotypes.

On the other hand, in spite of many positive association studies suggests that there is an influence of ACE genotype on athletic performance, there are also the studies have found no evidence for association described above [14,19]. Also the Nazarov [12] observed no association between the ACE genotypes in Russian athletes.

According to our results obtained, we found a significant difference on ACE I/D polymorphism between judo players and controls (p value 0.019). Our data support with the literature starting the presence of correlation in between ACE I/D polymorphism and sport performance.

Difficulties in the interpretation may be caused by a low occurrence of D/D in judo players (such an allele distribution would be most desirable due to the character of the performed efforts). On the other hand, a high occurrence of I/II genotype may be most desirable due to the duration of effort – in judo, fighting usually takes 5mins of pure fighting. Thus the obtained results indicate that in judo a genetic predispositions for longer fighting is more desirable than genetic predispositions to speed or power.

This conclusion seems to be confirmed by studies by Alvarez carried out on handball players [1]. Unfortunately, it is difficult to verify this hypothesis due to the insufficient number of surveys on the significance of genetic conditions in fighting sports (ACE gene has been examined only once in the context of judo, in a paper by Oh [13] but with no clear conclusions).

This genotype distribution can also be explained in the association with the physiology of the performed effort. Taking into account physiological aspects it is clear why I allele may influence endurance performance. Through improvements in substrate delivery, mainly by increase skeletal muscle glucose uptake [23] and adaptation of the enzymes responsible for glucose catabolism the efficiency of skeletal muscle is rising [22] with subsequent conservation of energy stores like glycogen storage [10].

Our investigation have proved the ACE I/D allele could be one of the factors influencing the elite endurance exercise performance. However, these results should be supported with more experimental data on ACE polymorphisms with large elite athletes, especially judo players.

CONCLUSIONS

1. The obtained results confirm the significance of the ACE gene as a useful genetic marker in sport selection in judo.
2. The research suggests that those most predisposed to judo are individuals with the allele distribution in the ACE gene that is most significant with regard to the duration of a fight (aerobic transformations), and not with regard to the character of the performed moves, as could be supposed.

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