ACTA MEDICA LITUANICA. 2009. Vol. 16. No. 1–2. P. 9–14

The angiotensin-converting enzyme gene insertion/deletion polymorphism in Lithuanian professional athletes

Valentina Ginevičienė[1, 2],

Vaidutis Kučinskas[1],

Jūratė Kasnauskienė[1]

[1] *Department of Human and Medical Genetics, Faculty of Medicine, Vilnius University, Lithuania*

[2] *Lithuanian Olympic Sports Centre, Vilnius, Lithuania*

Background. Human *ACE* gene was one of the first genes to be associated with human physical performance. Previous studies have indicated that *Alu* insertion and deletion polymorphism (I/D polymorphism) in the *ACE* gene may be associated with elite athlete status.

Materials and methods. *ACE* I/D polymorphism was investigated by PCR and gel electrophoresis in 561 Lithuanian professional athletes and in 174 samples from general population of Lithuania.

Results. Genotypes for athletes were identified as I/I 24.8%, I/D 47.2% and D/D 28% ($\chi^2 = 1.65$, $p = 0.19$) and in the population samples as I/I 24.1%, I/D 38.5% and 37.4% ($\chi^2 = 8.13$, $p = 0.004$). In comparison with the general Lithuanian population, the tested athletes had a lower frequency of *ACE* D allele. The results of this research contradict the results reported on other populations: the D/D genotype was found to be less frequent in all Lithuanian athlete groups than in general population, whereas, according to other researchers, the D/D genotype is more frequent in athletes. Athletes assigned to the endurance sports group had a lower frequency of the D/D genotype than athletes in the speed / strength group, whereas other researchers have reported a higher frequency of D/D genotype in endurance groups.

Conclusion. The *ACE* D/D genotype was less frequent in athletes than in the general population. Athletes of endurance sports had a higher frequency of D/D genotype than athletes assigned to the speed / strength sports group. These results do not support the results of other investigations conducted in other populations, in which the I allele of the *ACE* gene was found to determine superior endurance.

Key words: *ACE* I/D polymorphism, physical performance, elite athletes

INTRODUCTION

A number of investigators have indicated that individual variations in human physical performance reflect the interaction of both environmental factors, such as physique, biomechanical, physiological, metabolic, behavioral, psychological and social characteristics, as well as genetic element interaction. The effects of environmental factors on endurance performance are well documented, whereas few genetic loci of influence have been identified. One of them is the gene for angiotensin I-converting enzyme (*ACE*). Indeed, there is mounting evidence that the allelic variant of the gene encoding ACE (the *ACE* I/D polymorphism) is associated with human physical performance (1). The gene encoding ACE is located on the long arm of chromosome 17 (17q23). The gene is 21 kilobases (kb) long and comprises 26 exons and 25 introns. The polymorphism in intron 16 of the ACE gene is classified by the presence (insertion [I]) or absence (deletion [D]) of a 287-base-pair *Alu* sequence within the ***ACE*** gene (Fig. 1). With this notation, each I or D represents a single allele.

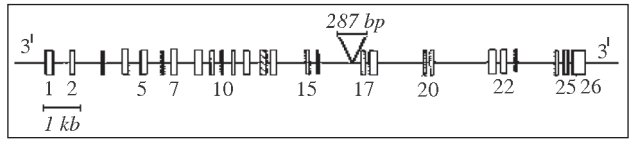

Fig. 1. Diagram of the *ACE* gene with exons (black boxes) and I/D polymorphism (indicated with arrows)

Because each gene has two alleles, there are three combinations of the genetic polymorphism: I/I, I/D, and D/D. The

Corespondence to: Vaidutis Kučinskas. Department of Human and Medical Genetics, Faculty of Medicine, Vilnius University, Santariškių 2, LT-08661 Vilnius, Lithuania. E-mail: vaidutis.kucinskas@santa.lt

distribution of these variants within the Caucasian population is roughly 25%, 50%, and 25%, respectively, being in approximate agreement with those within the Japanese population (2). Polymorphism occurs in the intron of the gene and therefore is thought to be non-functional; however, the presence of the D allele has been shown to be associated with a higher ACE activity than that of the I allele (3).

The angiotensin-converting enzyme plays an essential role in two physiological systems, one leading to the production of angiotensin II and the other to the degradation of bradykinin (Fig. 2) (4).

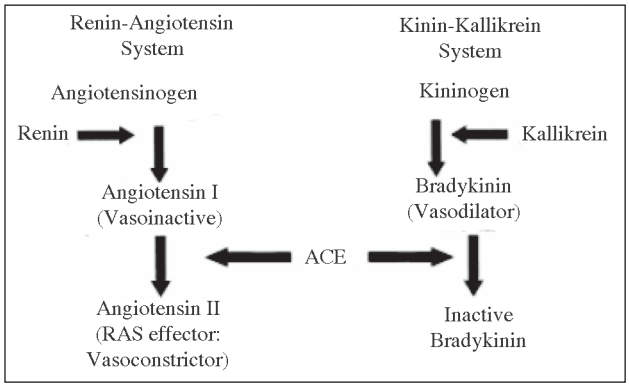

Fig. 2. The role of ACE in the kinin–kallikrein and renin–angiotensin systems (relationships among different proteins in this hormonal system) (4)

The importance of *ACE* in the renin–angiotensin system (RAS) has made it an often-studied candidate gene in relation to a number of cardiovascular endpoints, not to mention tissue growth phenotypes for tissue-specific renin–angiotensin systems. As such, *ACE* has become one of the most comprehensively studied genes with regard to exercise performance phenotypes (3, 5, 6). An excess of the I allele has been associated with some aspects of endurance performance, identified in elite British distance runners (7) and mountaineers (8). In addition, an excess of the I allele is present in Australian (9) and Croatian (10) rowers as well as in Spanish elite athletes (11). Conversely, an excess of the D allele has been reported amongst elite athletes in more power-oriented events such as short-distance swimming (7, 12) and sprinting (7). However, there has been debate as to the reproducibility of such associations. Several studies have failed to identify any association with elite endurance performance (5, 6, 13, 14). The mixed cohort examined by Karjalainen (14) included diverse sports such as long distance running, orienteering, cross country skiing and triathlon. The 192 athletes studied by Rankinen et al. (5) also included skiers, long and middle distance runners, cyclists and biathletes (the latter would also have to demonstrate more than a proficiency at rifle marksmanship). Such studies thus comprise individuals selected from diverse sporting disciplines, with a potential variation in standard, with events of varying duration and skill mix. In general, therefore, the association of the *ACE* genotype with sporting prowess is recognized in studies of elite athletes drawn from a single sporting discipline in whom the I and D alleles seem to associate with some aspects of endurance and power performance respectively (7–10, 12). The use of subjects from mixed disciplines might thus account for the lack of association reported in such study groups (5, 13, 14). The aim of our research was to identify the genotypes of elite Lithuanian athletes according to the ACE I/D polymorphism, to compare them to the general Lithuanian population and to find out if D allele in elite Lithuanian athletes is more frequent in endurance or the speed and strength sports group.

MATERIALS AND METHODS

In the present study, *ACE* gene I/D polymorphism was investigated in 561 Lithuanian professional athletes representing three functional groups: endurance (n = 71); strength and speed (n = 59), and team sports (n = 431) (Table 1), as well as in 174 samples from healthy unrelated volunteers of the general population of Lithuania. The athlete and control groups were all Caucasian Lithuanians.

Athletes were considered elite if they represented Lithuania in international competitions or at least took part in regional competitions with no less than 7 years of experience of participating in their sport. Informed written consent was obtained from all study participants.

Table 1. **Sports groups differentiated by separate sports**

Sports groups		Number of athletes
Group I (endurance)	Biathlon	5
	Pentathlon	4
	Cycling	11
	Skiing	12
	Swimming	13
	Academic rowing	21
	Track and field (long distance) athletics	5
	Total	71
Group II (strength and speed)	Wrestle	11
	Weightlifting	30
	Boxing	6
	Track and field (short distance) athletics	12
	Total	59
Group III (team sports)	Tennis	3
	Handball	14
	Field hockey	21
	Football	393
	Total	431
Total		561

Genomic DNA was extracted from peripheral blood leukocytes by the standard phenol / chloroform extraction method. Polymerase chain reaction was used to detect the I and D alleles in intron 16 of the *ACE* gene according to the method described by Rigat et al. (15), using the upstream

primer 5'-CTGGAGACCACTCCCATCCTTTCT-3' and the downstream primer 5'-GATGTGGCCATCACATTCGTCAGAT-3'. Amplification was performed for 35 cycles with denaturation, extension and annealing temperatures of 95 °C, 55 °C and 69 °C, respectively. The size of the amplified fragments was determined by 2% agarose gel electrophoresis, UVI gel documentation system (Fig. 3).

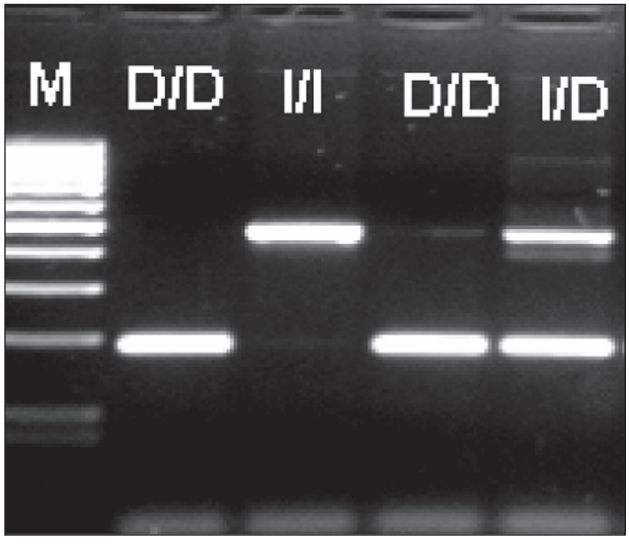

Fig. 3. Genotypes for the angiotensin-I-converting enzyme I / D polymorphism (D / D, I / D, I / I, M – DNA molecular-size standard (GeneRuler 100 bp)

Statistical analysis. The chi-square (χ^2) test was used to assess the fit of the observed genotype frequencies with the Hardy–Weinberg equilibrium. Continuous variables were expressed as means ± SD according to the genotypes (II, ID, DD). Genotype distribution and allele frequencies among the groups of athletes and controls were then compared by the χ^2 test. P values 0.05 or less were considered statistically significant.

RESULTS

ACE genotypes were determined in controls (174 individuals that did not exercise, from all ethnolinguistic groups of Lithuanians: East, West and South Aukstaiciai and North, South and West Zemaiciai) and professional athletes. Results of the study are summarized in Table 2.

Genotypes for athletes were identified as I/I 24.8%, I/D 47.2% and D/D 28% and in the population samples as I/I 24.1%, I/D 38.5% and D/D 37.4% ($\chi^2 = 8.13$, $p = 0.004$). The genotype frequencies in Lithuanian population show a significant departure from the Hardy–Weinberg equilibrium. In athletes, the I/D genotype is most frequent and the I/I and D/D genotypes are less common but have similar frequencies; however, this distribution is different in Lithuanian population where the D/D genotype is significantly more frequent than the I/I genotype.

When the I and D allele frequencies in groups of athletes are compared to those of the general Lithuanian population it can be seen that the frequency of the D allele in athlete groups is insignificantly lower, while the I allele is insignificantly more frequent in the control group. Differences in the genotype frequencies can also be seen when comparing different athlete groups (Table 2).

Among athletes, according to the literature (8, 13, 23), the I allele is more frequent in endurance groups. Several researchers were unable to experimentally prove an association between the I allele and endurance in athletes (6, 7, 25). In the present study, we also tested the differences in allele frequencies between the groups of athletes and the general population. We were unable to reveal any statistically significant difference in the frequency of I and D alleles between the study groups. The genotypes in athletes differed significantly from those in the control groups in general. The I/D genotype dominated in the athletes as compared with the general control population. The frequency of the D/D genotype among athletes was by about 9% lower than in non-athletes.

Table 2. **Allele and genotype frequencies of *ACE* gene I / D polymorphism in athletes and controls**

Sports groups	N	Allele frequencies %		Genotype frequencies %						χ^2	P value
		[I]	[D]	[I] [I]		[I] [D]		[D] [D]			
				O	E	O	E	O	E		
Group I (endurance)	71	47.9	52.1	28.1	22.9	39.4	49.9	32.3	27.2	3.13	0.07
Group II (strength and speed)	59	52.5	47.5	23.7	27.6	57.6	49.9	18.6	22.5	1.43	0.23
Group III (team sports)	431	47.9	52.1	24.4	23.0	47.1	49.9	28.5	27.1	1.37	0.24
Total	561	48.4	51.6	24.8	23.4	47.2	49.9	28	26.6	1.65	0.19
General population of Lithuanians (controls)	174	43.3	56.6	24.1	18.8	38.5	49.2	37.4	32.0	8.13	0.004

O – observed frequencies of individuals;

E – expected frequencies of individuals (according to Hardy–Weinberg equilibrium);

χ^2 – according to Hardy–Weinberg equilibrium;

p values of <0.05 were considered statistically significant.

Table 3. **Allele and genotype frequencies of *ACE* gene I/D polymorphism in footballers of different playing positions**

Footballer position	N	Allele frequencies %		Genotype frequencies %						χ^2	P value
		[I]	[D]	[I][I]		[I][D]		[D][D]			
				O	E	O	E	O	E		
Strikers	76	44.1	55.9	19.7	19.3	48.7	49.3	31.6	31.3	0.01	0.9
Defenders	108	47.7	52.3	25	22.7	45.4	49.9	29.6	27.4	0.89	0.3
Midfielders	147	48.6	51.4	27.2	23.6	42.9	49.9	29.9	26.4	2.97	0.8
Goalkeepers	30	51.7	48.3	30	26.8	43.3	49.8	26.7	23.4	0.53	0.4
Total	361	47.6	52.3	25.2	22.7	44.9	49.9	29.9	27.4	1.65	0.05

O – observed frequencies of individuals;

E – expected frequencies of individuals (according to Hardy–Weinberg equilibrium);

χ^2 – according to Hardy–Weinberg equilibrium;

p values of <0.05 were considered statistically significant.

The differences between the athletes and untrained people can be clearly seen when the control group is compared to the speed and strength and team-sports categories. The results of our study do not support the hypothesis of an association of the I allele with endurance as reported by other researchers (8, 13, 23).

Footballers were analysed as a separate group as they accounted for the major part of the athletes in the study. The genotype distribution of professional footballers playing in different positions is in agreement with the genotype distribution in all athletes (Table 3).

DISCUSSION

The obtained data show that the D/D genotype is less frequent in all athlete groups as compared to the general Lithuanian population. Elite endurance athletic performance is likely to depend on a complex interaction between cardiovascular and pulmonary functions, muscular metabolism and musculoskeletal adaptations. The D allele of *ACE* might confer an advantage for an elite performance in endurance sports on the basis of the following supporting evidence. The angiotensin-converting enzyme is widely expressed in human tissues, including skeletal muscles, and may play a metabolic role during exercise (16). In some studies, higher $V_{O_{2max}}$ levels, which indicate an improved oxidative capacity, were found to be related to *ACE* D allele (17, 18). The increased ACE activity associated with the D/D genotype may lead to an enhanced production of angiotensin II which is the predominant biological product of RAS and mediates many of the local effects of ACE on skeletal muscles. Angiotensin II is an indispensal factor in mediating vascular smooth muscle growth and affects capillary density in skeletal muscles (17). Moreover, angiotensin II exerts a direct hypertrophic effect on skeletal muscles, and AT1R-mediated angiotensin II is crucial for optimal overload-induced skeletal muscle hypertrophy (19). In addition, angiotensin II has been shown to regulate oxygen consumption and affect muscle energy expenditure (20). Data suggest that increased ACE activity leads not only to an augmented production of angiotensin II, but also to a reduction in angiotensin peptide which is known to have vasodilating effects (16). Thus, it is conceivable that the D allele of *ACE* positively contributes to elite endurance performance by its involvement in the fine-tuning of the levels of both angiotensin II and angiotensin. There is also evidence that angiotensin II is involved in cell growth and that the D allele is associated with ventricular hypertrophy. Accordingly, several studies reported an association between the D allele and the left ventricular mass of athletes (21). Ashley et al. have recently reported that in ultramarathon runners, homozygotes for the D allele exhibited an increase in sympathovagal balance, resulting in greater sympathetic activation after the race that may have served to limit the decline in the left ventricular function (22). This effect might be associated with improved high-level performance and may contribute to athletic success. We, like others, undertook the population testing approach in the present study in an attempt to correlate *ACE* I/D polymorphism with elite athletic performance. Through this approach, positive results were achieved, i. e. an association has been found with either the I or the D allele which occurred more frequently in elite athletes than in the control population. To date, controversial results have been reported regarding the association of *ACE* I/D polymorphism with athletic ability. While several studies have suggested that the two functional alleles of the human *ACE* gene differ in their effects on athletic ability, the I allele favouring endurance performance (8, 13, 23) and the D allele promoting strength and speed events (8, 13), a number of other reports have found no association between *ACE* polymorphism and elite athletic performance (6, 7, 25). These inconsistencies represent some of the difficulties in the interpretation of association studies and may be attributed to the experimental design, the varying definition of elite athlete phenotype or the type of study cohort which contained athletes from a heterogeneous range of sporting disciplines (6, 7, 25). There is a disagreement between our findings and other reports showing a higher frequency of the I allele in endurance athletes (8, 9, 12, 23); however, since the frequencies of the *ACE* I/D alleles vary considerably among different control populations with varying the genetic background, we cannot exclude the possibility that the allele frequencies in sports groups are associated with allele fre-

quencies of the general Lithuanian population, although the D allele is less frequent among elite athletes. Finally, it is important to bear in mind that the interpretation of a positive association can be either that the *ACE* I/D polymorphism itself is responsible, or that it is in a linkage disequilibrium with another functional variant in an adjacent gene which is in fact responsible for the observed associations with the *ACE* genotype (6, 25). Indeed, it has been proposed that the higher ACE activity associated with the *ACE* D/D genotype is perhaps caused by an *Alu*-associated transcription silence or by an unidentified variant in the promoter region of the *ACE* gene which is in a strong allelic association with the Alu insertion / deletion polymorphism (24, 25). Our study groups were not large because of limitations imposed by the small number of elite athletes available for study in each sports discipline. In spite of the relatively small number of participants, our study groups demonstrated unique and clearly distinctive phenotypes. Each group contained elite level athletes from a well-defined sports category having known prime determinants for success. In such sample sizes, true positive associations cannot be masked.

CONCLUSIONS

The *ACE* I/D polymorphism in the groups of tested athletes differed from that of the general population, the D/D genotype being less frequent in athletes than in the general population. Athletes of endurance requiring sports had a higher frequency of the D/D genotype as compared to athletes assigned to the speed and strength sports group. These results do not support the results of other investigations conducted in other (non-Lithuanian) populations in which superior endurance was found to be determined by the I allele of the *ACE* gene.

Received 23 January 2009
Accepted 14 April 2009

References

1. Ohno H, Kizaki T, Suzuki K et al. Is angiotensin I-converting enzyme I / D polymorphism associated with endurance performance and / or high altitude adaptation? Adv Exerc Sports Physiol 2005; 11: 41–54.
2. Jones A, Montgomery HE, Woods DR. Human performance: a role for the ACE genotype? Exerc Sport Sci Rev 2002; 30: 184–90.
3. Thompson WR, Binder-Macleod SA. Association of genetic factors with selected measures of physical performance. Phys Ther 2006; 86: 585–91.
4. Sayed-Tabatabaei FA, Oostra BA, Isaacs A et al. ACE polymorphisms. Circulation Res 2006; 98: 1123–33.
5. Rankinen T, Wofarth B, Simoneau JA et al. No association between the angiotensin-converting enzyme ID polymorphism and elite endurance athlete status. J Appl Physiol 2000; 88: 1571–5.
6. Kenyans SRA, Moran C et al. No association between angiotensin-converting enzyme (ACE) gene variation and endurance athlete status in comparative biochemistry and physiology. Part A: Molecular & Integrative Physiology 2005; 141: 169–75.
7. Myerson S, Hemingway H, Budget R et al. Human angiotensin I-converting enzyme gene and endurance performance. J Appl Physiol 1999; 87: 1313–6.
8. Montgomery HE, Marshall RM, Hemingway H et al. Human gene for physical performance. Nature 1998; 393: 221–2.
9. Gayagay G, Yu B, Hambly B et al. Elite endurance athletes and the ACE I allele – the role of genes in athletic performance. Hum Genet 1998; 103: 48–50.
10. Jelakovic B, Kuzmanic D, Milicic D et al. Influence of angiotensin-converting enzyme (ACE) gene polymorphism and circadian blood pressure (BP) changes on left ventricle (LV) mass in competitive oarsmen. Am J Hypertens 2000; 13: 182.
11. Alvarez R, Terrados N, Ortolano R et al. Genetic variation in the renin–angiotensin system and athletic performance. Eur J Appl Physiol 2000; 82: 117–20.
12. Woods D, Hickman M, Jamshidi Y et al. Elite swimmers and the D allele of the ACE I / D polymorphism. Hum Genet 2001; 108: 230–2.
13. Taylor RR, Mamotte CDS, Fallon K, Bockxmeer FM. Elite athletes and the gene for angiotensin-converting enzyme. J Appl Physiol 1999; 87: 1035–7.
14. Karjalainen J, Kujala UM, Stolt A et al. Angiotensinogen Gene M235T polymorphism predicts left ventricular hypertrophy in endurance athletes. J Am Coll Cardiol 1999; 34: 494–9.
15. Rigat B, Hubert C, Corvol P, Soubrier F. PCR detection of the insertion / deletion polymorphism of the human angiotensin-converting enzyme gene (DCP1) (dipeptidyl-carboxypeptidase 1). Nucl Acids Res 1992; 20: 1433.
16. Jones A, Woods D. Skeletal muscle RAS and exercise performance. Int J Biochem Cell Biol 2003; 35: 855–66.
17. Rankinen T, Perusse L, Gagnon J et al. Angiotensin-converting enzyme ID polymorphism and fitness phenotype in the HERITAGE Family Study. J Appl Physiol 2000; 88: 1029–35.
18. Zhao B, Moochhala S, Tham S et al. Relationship between angiotensin-converting enzyme ID polymorphism and VO2max of Chinese males. Life Sci 2003; 73: 2625–30.
19. Gordon S, Davis B, Carlson C, Botth F. Ang II is required for optimal overload-induced skeletal muscle hypertrophy. Am J Physiol Endocrinol Metab 2001; 280: 150–9.
20. Cassis L, Helton M, English V, Burke G. Angiotensin II regulates oxygen consumption. Am J Physiol Regul Integr Comp Physiol 2002; 282: 445–53.
21. Hernandez D, de la Rosa A, Barragan A et al. The ACE / DD genotype is associated with the extent of exercise-induced left ventricular growth in endurance athletes. J Am Coll Cardiol 2003; 42: 527–32.

22. Ashley EA, Kardos A, Jack ES et al. Angiotensin-converting enzyme genotype predicts cardiac and autonomic responses to prolonged exercise. J Am Coll Cardiol 2006; 48: 523–31.
23. Collins M, Xenophontos SL, Cariolou MA et al. The ACE gene and endurance performance during the South African Ironman Triathlons. Med Sci Sports Exerc 2004; 36: 1314–20.
24. Rieder M, Taylor S, Clark A, Nickerson D. Sequence variation in the human angiotensin-converting enzyme. Nat Genet 1999; 22: 59–62.
25. Amir O, Amir R, Yamin C et al. The ACE deletion allele is associated with Israeli elite endurance athletes. Exper Physiol 2007; 5: 881–6.

Valentina Ginevičienė, Vaidutis Kučinskas, Jūratė Kasnauskienė

LIETUVOS PROFESIONALIŲ SPORTININKŲ ANGIOTENZINĄ KONVERTUOJANČIO FERMENTO GENO I/D POLIMORFIZMO TYRIMAI

Santrauka

Įvadas. ACE genas yra vienas iš genų, kurio įtaka žmogaus fiziniam pajėgumui buvo išaiškinta pirmiausiai. Ankstesniais tyrimais nustatyta, kad šio geno Alu sekos intarpo buvimas ar nebuvimas (I/D polimorfizmas) gali būti susijęs su profesionaliu sportiniu pajėgumu. Tačiau *ACE* I/D polimorfizmo tyrimų, atliktų įvairiose populiacijose, rezultatai yra prieštaringi.

Medžiaga ir metodai. *ACE* I/D polimorfizmas ištirtas 561 didelio meistriškumo Lietuvos sportininkui bei 174 Lietuvos populiacijos asmenims. Sportininkai buvo suskirstyti į tris grupes pagal jų sporto šakas. Genominė DNR buvo išskirta iš tiriamųjų asmenų periferinio kraujo leukocitų, PGR metodu pagausinti *ACE* fragmentai buvo vertinami pagal fragmento dydį.

Rezultatai. Tarp sportininkų *ACE* I/I genotipas buvo nustatytas 24,8%, I/D – 47,2% ir D/D – 28% ($\chi^2 = 1,65$, $p = 0,19$), bendroje Lietuvos populiacijoje I/I – 24,1%, I/D – 38,5% ir 37,4% ($\chi^2 = 8,13$, $p = 0,004$). ACE D alelio dažnis didelio meistriškumo Lietuvos sportininkų grupėje retesnis nei bendroje populiacijoje. Atitinkamai D/D genotipas nustatomas rečiau sportininkų nei bendrosios populiacijos grupėje (kitų šalių tyrėjų duomenimis, D/D genotipas dažnesnis sportininkų grupėje). Skirtingai nei kitų šalių mokslininkų duomenimis, mes nustatėme, kad D/D genotipas dažnesnis ištvermės reikalaujančių sporto šakų nei greičio ir jėgos sporto šakų Lietuvos sportininkų grupėje.

Išvados. *ACE* I/D polimorfizmo dažnis didelio meistriškumo Lietuvos sportininkų grupėje skiriasi nuo bendrosios populiacijos: D/D genotipas sportininkų grupėje yra retesnis nei bendroje populiacijoje. Ištvermės reikalaujančių sporto šakų sportininkų D alelis dažnesnis nei greičio ir jėgos sporto šakų sportininkų. Mūsų duomenys nesutampa su kitų šalių mokslininkų publikuotais darbais, kuriuose nustatyta, kad geresnę žmonių fizinę ištvermę lemia *ACE* geno I alelis.

Raktažodžiai: *ACE* I/D polimorfizmas, fizinis pajėgumas, didelio meistriškumo sportininkai