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ANALYSIS OF THE VARIETY OF HUMAN GENOME LOCI ASSOCIATED WITH FAST AND LONG-LASTING ADAPTATION TO THE LOAD OF PHYSICAL ACTIVITY

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VILNIAUS UNIVERSITETAS

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ŽMOGAUS GENOMO SRIČIŲ, SUSIJUSIŲ SU GREITA IR ILGALAIKE ADAPTACIJA FIZINIAM KRŪVIUI, ĮVAIROVĖS ANALIZĖ

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INTRODUCTION

Physical capacity is a complex, inheritable and quantitative trait, the phenotypic expression of which could be influenced by both many genes and environmental factors. The determination of genetic factors provides basis for the evaluation of differences in individual physical development as well as in functional capacity. It helps to determine whether an athlete's body is well fit for a certain activity and predict its athletic development. Many efforts have been directed towards determination of the candidate genes affecting physical capacity (Ahmetov et al., 2009; Bray et al., 2009). Irrespective of a large number of candidate genes (there are 214 autosomal genes and genes that could be associated with quantitative features, 7 genes in the X chromosome and 18 mitochondrial DNA genes known) each candidate gene exercises only a partial influence on the athletic phenotype (Bray et al., 2009). Active search for genes and genetic areas, most adequately reflecting the physical potential of an athlete, is being continued. There is an increasing amount of data concerning the association of gene polymorphism with athletic capacity. It offers the possibility to further investigate gene polymorphisms predisposing an athlete towards specific sporting activities. As a result the most suitable sport can be recommended for an athlete, and the most appropriate personalized training regime is becoming more realistic in an attempt to achieve elite status. Bodily response to physical strain depends on genetic makeup and can significantly impact not only the physical capacity but also health, life quality in senior years and, possibly, one's life expectancy (Thompson et al., 2006; Lippi et al., 2009).

Aim of the study:

To evaluate the features of the genetic variety of Lithuanian athletes according to the DNA markers associated with individual responses to physical strain.

Main tasks of the study:

1. To select genetic markers for the research associated with physical capacity.
2. To research and evaluate the frequency and distribution of the alleles of the candidate genes associated with physical capacity between the sports groups and the control group.

3. To carry out analysis of genetic and phenotypic features of the biobase of Lithuanian elite athletes according to the markers related to the physical capacity by using bioinformatics and biostatistical analysis methods and to evaluate the association of the athletic phenotypes with genotypes according to the variants of the researched candidate genes associated with physical capacity.
4. To compare the determined genetic variance parameters of the Lithuanian population and those of athletes with analogous parameters of other populations.
5. To create and accumulate a DNA biobase of Lithuanian elite athletes by collecting their phenotype and other data, comprehensively characterized according to the physical development and athletic capacity phenotypes.

Relevance and novelty of the research

This research is devoted to the issue of the effect of genetic factors on the components of physical capacity as well as to the exploration of a possibility to implement new research methods in Lithuania's practice and to produce recommendations on how to improve athletic results. The knowledge concerning the individual genomes of the athletes is especially important for sports theory, practice and medicine. Significance and relevance of our work is supported by the fact that understanding of molecular physiological processes through genetic analysis is essential to the creation of a perfect health care system for athletes in Lithuania. Moreover, it would enable to achieve implementation of personalized training programs. In addition to improving the training process, the results of genetic research can also help choose the most appropriate physical loads for the prevention and treatment of chronic diseases.

We have accumulated a sample of Lithuanian elite athletes, who were investigated according to a phenotype, while absence of such biosamples and data in this biobase makes further genomic research of Lithuanian athletes impossible. During research bioinformatics analysis was carried out, and the strongest candidate genes related to physical fitness were selected. It was for the first time in Lithuania that the elite athletes were investigated according to allelic distribution of 6 candidate gene variants most associated with physical capacity.

Statements to be defended

1. For the analysis of the Lithuanian athletes included in the biobase, the candidate genes and their polymorphisms were associated with the physical capacity: *ACTN3 (*α-actinin-3 gene) c.1747C>T (p.R577X, rs1815739), *ACE* (angiotensin converting enzyme gene) I/D (*Alu* sequence insertion/deletion); *AGTR1* (angiotensin II type 1 receptor) c.1166A>C, (rs5186), *PPARGC1A* (peroxysome proliferator activated δ receptor coactivator 1α gene) c.1444G>A, (p.Gly482Ser, rs8192678); *PPARA* (peroxysome proliferator activated receptor gene) c.2528G>C (rs4253778); *PPARG* (peroxysome proliferator activated γ receptor gene) c.34C>G, (p.Pro12Ala, rs1801282).
2. The genetic diversity of the physical capacity in Lithuania's population has a pattern manifested by variation in the allele/genotype frequencies of the selected candidate gene markers in the Lithuanian athlete groups and general population. Each investigated group of athletes had a typical genotype/allele combination.
3. The indexes of physical development and functional capacity of the Lithuanian athletes correspond to the elite levels. Inherited qualities and adaptation to physical loads of the athletes can be assessed by statistical analysis of phenotypic indexes. The genotypes of the gene variants investigated have different levels of influence on the physical capacity of males and females and are statistically significantly associated with phenotypic indexes.
4. *ACTN3* c.1747C>T polymorphism T/T genotype athletes adapt well to physical strain and achieve high results in any sport discipline; *ACE* I/D polymorphism D/D genotype Lithuanian athletes show typically higher endurance, while speed and power is characteristic of I/I genotype athletes. Athletes with any *AGTR1* c.1166A>C polymorphism genotype have characteristically higher endurance as well as speed and power. *PPARGC1A* c.1444G>A polymorphism G/G genotype and *PPARG* c.34C>G polymorphism C/C genotype athletes have typical endurance, whereas *PPARA* c.2528G>C polymorphism C/C genotype Lithuanian athletes possess typical speed and power.
5. Inheritance plays a greater role in case of speed and strength qualities compared to those of endurance.

MATERIALS AND METHODS

Study population

A DNA biobase was created at the Molecular Genetics Laboratory of the Department of Human and Medical Genetics, the Faculty of Medicine, Vilnius University.

During the period from 2006 to 2010 DNA samples of 630 Lithuanian athletes were collected. The athletes were stratified into three groups according to their physical work duration and the specificity of their sporting discipline. The first group consisted of the athletes developing their endurance and involved in high power and aerobic type of physical strain sport disciplines (60 males and 17 females, average age 24.8±6.5 years). They included skiers (n=12), (road) cyclists (n=12), biathlonists (n=5), track and field athletics sportsmen (long distance runners) (n=9), modern pentathlonists (n=4), swimmers (n=13), rowers (academic rowing) (n=9) as well as kayak and canoe rowers (n=13). The second group was made up of anaerobic capacity *i.e.* athletes developing speed and power (46 males and 5 females, average age 22.1±5.9 years). These included track and field sportsmen (sprinters, jumpers and throwers) (n=20) and weightlifters (n=31). The third group was comprised of aerobic and anaerobic athletes cultivating games and duel sports (51 male and 14 females, average age 20.9±5.7 years). These included wrestlers (n=10), tennis players (n=3), handball players (n = 14), footballers (n=32) and boxers (n=6).

In total, the sample consisted of the world and European championship medalists (elite, n=43), Olympic reserve athletes (sub-elite, n=83) and members of the Lithuanian National Youth Team (non-elite, n=504). In addition, 250 (167 males and 83 females) randomly selected non-related control group people were researched who were chosen from 6 Lithuanian ethno linguistic groups, the parents and grandparents of whom lived in the same region of Lithuania). These population samples were collected and DNA was extracted during the period from 1994 to 1996 at the Department of Human and Medical Genetics of the Faculty of Medicine, Vilnius University.

Phenotypic data

Analysis of the phenotypic indexes of the Lithuanian athletes was carried out in 2007–2009 in the Vilnius Sports Medicine Centre and Vilnius Pedagogical University Sports Laboratories. For the evaluation of the physical potential of the 193 athletes, whose DNA is stored in the biobase of the Department of Human and Medical Genetics, the following main phenotypic physical development indexes were used: height (cm), body mass (kg), fat mass (FM) and muscle mass (MM) (kg), body mass index (BMI), muscle fat mass index (MFMI), left and right handgrip strength (kg), vital capacity (VC) (Norton K et al., 1996). Physical capacity was evaluated according to the short-term explosive muscle power (STEMP) according to the Bosco methods (Bosco et al., 1982; Bosco C., 2000); anaerobic alactic maximum power (AAMP) was estimated by a stair climbing test proposed by Margaria (Margaria et al., 1966; Nedeljkovic et al., 2007). CVS and pulmonary system (aerobic capacity) function was determined using Roufier index (RI). 149 athletes were evaluated according to the maximum oxygen consumption (VO_2max, ml/min/kg) (Norton, 1996).

Genetic testing

DNA of the Lithuanian athletes was extracted from peripheral blood leukocytes by using phenol-chlorophorm method. DNA purity and concentration was measured using a spectrophotometer. This research involved analysis of 6 candidate gene markers.

The markers chosen within the candidate gene, their characteristics, research methods, oligonucleotide primer sequences are presented in Table 1.

The DNA fragment researched was amplified by using polymerase chain reaction (PCR) method. The PCR product was evaluated using electrophoresis in 2% agarose gel. *ACE* I/D polymorphism analysis was performed using PCR method. Single nucleotide polymorphisms (*ACTN3* C/T, *AGTR1* A/C, *PPARGC1A* G/A, *PPARA* G/C, *PPARG* C/G) were analyzed by using restriction-fragment length polymorphism (PCR-RFLP) method: the DNA fragments obtained by PCR were cut using restriction endonucleases (Table 1).

Table 1. Candidate gene markers, characteristics and research method

Gene	*Chromosomal position*	*NCBI ref. SNP ID*	*SNP*	*Genotyping / Methods*	*Primers*
ACTN3 (α-actinin-3 gene)	11q13-q14	rs1815739	c.1729C>T (p.Arg577X) exon 16	RFLP (*DdeI*)	F 5'-CTGTTGCCTGTGGTAAGTGGG -3'
					R 5'-TGGTCACAGTATGCAGGAGGG -3'
ACE (angiotensin-1-coverting enzyme gene)	17q23	rs1799752	I/D (insertion/deletion) of a 287-base-pair *Alu* sequence	PCR	F 5'-CTGGAGACCACTCCCATCCTTTCT-3'
					R 5'-GATGTGGCCATCACATTCGTCAGAT-3'
AGTR1 (angiotensin II type 1 receptor gene)	3q21-q25	rs5186	c.1166A>C 3'UTR	RFLP (*AluI*)	F 5'-AGAAGCCTGCACCATGTTTTGAG-3'
					R 5'-TAAATCGTTAGAGGAGCAACAGG-3'
PPARA (peroxisome proliferator activated receptor α gene)	22q12-q13.1	rs4253778	c.2528G>C intron 7	RFLP (*TagI*)	F 5'-ACAATCACTCCTTAAATATGGTGG -3'
					R 5'-AAGTAGGGACAGACAGGACCAGTA -3'
PPARG (peroxisome proliferator activated receptor γ gene)	3p25	rs1801282	c.34C>G, (p.Pro12Ala) exon 2	RFLP (*Bsh1236I*)	F 5'-GCCAATTCAAGCCCAGTC -3'
					R 5'GATATGTTTGCAGACAGTGTATCAGTGAAG GAATCGCTTTCCG -3'
PPARGC1A (peroxisome proliferator activated receptor γ coactivator I α)	4p15.1	rs8192678	c.1444G>A (p.Gly482Ser) exon 8	RFLP (*MspI*)	F 5'-TTGTTCTTCCACAGATTCAGAC -3'
					R 5'-GAAAAGGACCTTGAACGAGAG -3'

PCR – polymerase chain reaction; RFLP – restriction-fragment length polymorphism (restriction endonuclease in the brackets); F (*forward*) – upstream primer, R (*reverse*) – downstream primer; 3'UTR - three prime untranslated regions

Restriction reactions were evaluated in a 2% agarose or 8% polyacrilamyde gel (in case of the *ACTN3* C/T polymorphism). Electrophoretogram was analyzed, and the results evaluated and interpreted

Statistical analysis

Deviation from the Hardy-Weinberg equilibrium was statistically evaluated. Chi squared criterion (χ^2) was used with the significance level at 0.05. The average differences for each genotype of Lithuanian athletes' physical development and functional capacity indexes were evaluated by using single factor dispersion analysis method (ANOVA). The influence of genotypes on the physical development and functional capacity was assessed by creating linear regression models (Brooker, 2005). Statistical analysis package SPSS version 13.0 was used for the calculation of the results.

RESULTS AND DISCUSSION

The study assessed the impact of genetic variation of DNA markers according to six candidate genes onto individual responses to the physical strain in a group of Lithuanian professional athletes according to the guidelines by associating genotype to phenotype (Chanock et al., 2007). In order to ensure the proper storage and accessibility of the DNA samples, the biobase was created at the Department of Human and Medical Genetics of the Faculty of Medicine, Vilnius University, where molecular and phenotypic information of the athletes studied was stored. The candidate genes were selected as a result of bioinfomation analysis.

Analysis of scientific literature in the databases revealed more than 80 candidate genes related to the phenotypes of human physical fitness. These genes can be included into further studies of genetic and genomic analysis of the Lithuanian athletes, aiming to select a set of alleles, associated with physical fitness, for the purpose of designing a genotyping microarray.

At present, based on the analysis of scientific publications several potential biomarkers known to have influence on human physical capacity were chosen for the genetic

analysis of the Lithuanian athletes and general population: *ACTN3* (α-actinin-3) gene c.1747C>T (p.R577X) polymorphism, *ACE* (angiotenstin converting enzyme) gene *Alu* sequence (I/D) indel, *AGTR1* (first type angiotensin II receptor) gene c.1166A>C polymorphism, *PPARGC1A* (peroxisome proliferator activated δ receptor coactivator 1α) gene c.1444G>A, (p.Gly482Ser) polymorphism, *PPARA* (peroxisome proliferator activated α receptor) gene c.2528G>C, and *PPARG* (peroxisome proliferator activated γ 2 receptor gene) c.34C>G, (p.Pro12Ala) polymorphisms.

It is feasible to research the impact of the selected polymorphisms onto the human physical capacity of professional athletes for they have the most developed physical fitness indexes. The control group served as a reference for comparison whether the genotype and allele frequencies differ between the athletes and general non-exercising population. Since the physical phenotypic characteristics were not measured, the athletes were grouped into the categories based on their sporting achievements since professionalism in sports (winning in the world and Eurpean championships) is achieved as a result of genotype-phenotype interaction. Environment and genetic background are also important for non-elite sportsmen (e.g. members of the National Youth Team) athough their achievements are to be decided in the future. Regardless of young age of non-elite athletes, the genetic study revealed a promising insight into the relationship between the genetic background and achievements in sport.

Phenotypic indexes of Lithuanian athletes: analysis and evaluation

Fast twitch fibers are more dominant than slow endurance ones in the muscles of speed and power-oriented athletes (Stewart and Rittweger, 2006; Dadelienė, 2008; Lippi et al., 2009). Aerobic energy production takes place in the muscles of endurance-oriented athletes, slow endurance fibers are activated since they can produce more energy during the aerobic reactions. Success in achieving the task during athletic games depends very much on frequent repetition of “explosion-type” activities, whereas an aerobic and anaerobic supply of the energy is taking place during physical training (Stewart and Rittweger, 2006; Lippi et al., 2009). The organism of an athlete adapts differently in response to training and to participation in sports competitions (Stewart and Rittweger, 2006; Dadelienė, 2008).

Our study included professional athletes practicing in a variety of sports. The average indexes of physical fitness and capacity of all the athletes studied were as follows: average body mass 73.5±14.5 kg, height 178.5±9.4 cm, fat mass 8.4±3.4 kg, muscle mass 39.5±9.1 kg, body mass index (BMI) 22.8±3.4 kg/m^2, muscle fat mass index (MFMI) 5.2±1.7, lung volume (LV) 4.9±0.4 l. The measures of anaerobic capacity were the following ones: short term explosive muscle power (STEMP) 1750.1±498.8 W, anaerobic alactic muscle power (AAMP) 1151.4±296.5 W. Cardiovascular and respiratory systems were characterized by average Roufier index (RI) 4.5±2.9, average aerobic capacity index or VO_2max 55.5±10.5 ml/min/kg.

The distribution of indexes of physical development and capacity in Lithuanian athletes in groups according to sports category and duration of physical load are presented in Table 2. Phenotypic characteristics were compared by using one way analysis of variance (ANOVA). Their average values were specific for each sport category, except VO_2max, and statistically significantly different among them ($p<0.05$).

Table 2. Distribution of indexes of physical development and capacity in Lithuanian athletes in groups according to sports category and duration of physical load

Sports group / *Phenotype*	***Group I (n=77)***	***Group II (n=51)***	***Group III (n=65)***
Height, cm	181.9±8.4*	178.3±9.6*	174.5±8.8*
Weight, kg	76.0±12.5*	81.1±16.3*	64.4±10.0*
BMI, kg/m^2	22.8±2.4*	25.4±4.1*	21.0±2.3*
Fat mass, kg	7.9±2.2*	9.3±4.7*	8.1±2.9*
Muscle mass, kg	41.2±8.6*	44.6±8.9*	33.6±6.1*
MFMI	5.5±1.5	5.5±2.3	4.5±1.1
LV, l	5.6±0.4*	4.7±0.6*	4.4±0.2*
Right handgrip strength, kg	47.9±9.9*	52.0±9.1*	39.6±8.4*
Left handgrip strength, kg	47.0±9.3*	50.9±9.2*	38.3±9.6*
STEMP, W	1763.9±484.8*	1995.7±497.3*	1541.0±425.9*
AAMP, W	1210.1±286*	1238.5±240.9*	1016.6±220.2*
Roufier index	3.3±2.6*	5.7±3.0*	4.9±3.0*
VO_2max, ml/min/kg	55.2±9.3	58.9±10.7	54.1±11.6

The data are presented as means ± standard deviations. * $p<0.05$ indicates significant differences between the sport groups. Group I – representatives of endurance sports; group II – representatives of speed and power demanding sports; group III – representatives of game sports and duels; BMI – body mass index; MFMI – mussle fat mass index; LV – life lung volume; STEMP – short term explosive muscle power; AAMP – anaerobic alactic muscle power; VO_2max – maximum oxygen consumption.

The athletes involved in speed and power demanding sports had higher values of muscle mass, palm strength, STEMP and AAMP compared to those cultivating endurance, team

and duel sports. These results can be attributed to the excellence and talent of the athletes. Our results are in agreement with the opinion of the other scientists who pointed out that the athletes in speed and power demanding sports have higher measures of anaerobic capacity such as STEMP and AAMP than those who practice endurance demanding sports (Dadelienė, 2008). According to many studies, maximum anaerobic capacity is strongly related to body volume, especially to the muscle mass (Huygens et al., 2004) which is also evident in our results.

The STEMP and AAMP and palm strength indexes are higher in the athletes of larger weight but smaller fat mass. The lung volume in endurance group was higher compared to other sport categories. According to literature, the LV is genetically predisposed (h^2 in between 43 and 78) (Chatterjee and Das, 1995). In athletes developing the aerobic endurance the LV increases. If the training stops, it starts to decrease (Dadelienė, 2008). The results of the Roufier test of cardiovascular and respiratory system were lower in the endurance athletes than in the other groups, which indicates good acquired fitness and aerobic capacity.

Table 3. Distribution of indexes of physical development and capacity in Lithuanian athletes in groups according to sports category, fully characterizing the sporting potential and development

Sports group / *Phenotype*	*Elite athletes (n=43)*	*Sub-elite athletes (n=52)*	*Non-elite athletes (n=98)*
Height, cm	180.8±5.9	182.2±2.0	174.7±2.9
Weight, kg	77.9±6.3	75.8±2.9	68.5±4.3
BMI, kg/m^2	23.6±0.8	22.7±0.5	22.3±0.9
Fat mass, kg	7.5±0.8	9.6±0.2	7.9±0.8
Muscle mass, kg	41.9±0.5	41.1±1.6	36.7±2.4
MFMI	6.2±0.5	4.9±0.7	4.9±0.3
LV, l	5.6±0.5	5.2±0.2	4.4±0.5
Right handgrip strength, kg	48.3±5.0	44.4±2.7	45.3±2.5
Left handgrip strength, kg	46.6±5.6	43.6±3.2	44.0±3.3
STEMP, W	1875.9±184.2	1842.9±162.7	1625.8±159.2
AAMP, W	1256.4±107.2	1170.7±63.9	1106.5±48.1
Roufier index	2.3±0.7	5.5±1.1	4.9±0.6
VO_2max, ml/min/kg	55.3±3.1	57.8±3.7	54.8±1.3

The data are presented as means ± standard deviations. BMI – body mass index; MFMI – mussle fat mass index; LV – life lung volume; STEMP – short term explosive muscle power; AAMP – anaerobic alactic muscle power; VO_2max – maximum oxygen consumption.

All the measures of physical development and capacity are adequate for high professionalism in sports. Descriptive summary of the phenotypic characteristics of the Lithuanian elite athletes practicing in different sports is presented in Table 3. Phenotypic values in each group were specific to the sport category, fully characterizing the sporting potential and development. The Lithuanian elite athletes had higher palm strength, STEMP and AAMP than sub-elite and non-elite ones indicating high sports qualification (Table 3). The organism of an individual athlete has a unique pattern of growth of the functional capacity.

ACTN3 C/T polymorphism association analysis: results and discussion

Many genes influence adaptation to physical strain, *ACTN3* (α-actinin-3 gene) being one of them. Many scientific studies of numerous populations suggest *ACTN3* as a strong gene candidate determining human physical capacity.

There are two isoforms of α-actinin protein in skeletal muscle: α-actinin-2 (ACTN2) and α-actinin-3 (ACTN3). ACTN2 is found in all the muscle fiberes, while ACTN3 is present only in fast twitch (II type) muscle fibers. Deficiency of ACTN3 is caused by a single nucleotide change c.1747C>T (p.R577X, rs1815739) in exon 16 of *ACTN3* gene. *ACTN3* C/T polymorfism is not related to muscle pathology regardless of the absense of ACTN3 protein in fast twitch muscle fibers of individuals carrying T/T genotype (Ahmetov and Rogozkin, 2009; Clarkson et al., 2005; Collins et al., 2009). According to literature and genomic databases, the frequency of homozygous T/T genotype of *ACTN3* is different in different populations: in African populations it is only 1%, in Europeans it makes about 18%, whereas in Asian populations exceeding up to 25% (dbNCBI, North, 2008).

We found that in general Lithuaninan population (n=250) the frequency of *ACTN3* T/T genotype was 10.4%, it was also similar in athletes, 10.6%. The genotype and allele frequencies in the Lithuanian athletes and control group are presented in Table 4. In our study the distribution of *ACTN3* genotype deviates from Hardy-Weinberg equilibrium (HWE). There were more athletes carrying *ACTN3* C/T heterozygous genotype, however, there were no deviations from expected frequencies in the control group (Table

4). This finding implies that intensive training and other factors determine natural selection of athletes because only the best fitted individuals remain in sports.

N. Yang and collegues were the first who reported in 2003 that in the highest level sprinter group the *ACTN3* T/T genotype is very rear (Yang et al., 2003). In other studies it was revealed that in speed and power demanding sports the best results were achieved by the atletes who have *ACTN3* C/C and C/T genotypes (Yang et al., 2003; MacArthur et al., 2008; Moran et al., 2007). However, we found that there were more *ACTN3* T/T individuals (15.7%) in speed and power-oriented sports compared to other sport categories and controls ($p>0.05$). It is possible that a lack of ACTN3 in fast twitch fibers is compensated.

D. G. MacArthur and colleagues in 2008 created a genetically modified strain of mice without functional *ACTN*3 gene in muscles. More enzymes were found connected to aerobic metabolism in the muscle fibers of such mice (MacArthur et al., 2008). Investigating polymorphism influence of this group onto the human muscle activity and physical capacity revealed that higher than expected frequency of heterozygous genotype was in high-level sprinters and lower than expected in endurance oriented athletes (MacArthur et al., 2008). We did not observe this in our group; however, we observed the opposite in males: in speed and power sports there were fewer heterozygous individuals than in the endurance group. Similarly to other researchers we found that the distribution of genotype frequencies was different in men and women athletes suggesting different influence of *ACTN3* C/T polymorphism to the physical capacity of different genders.

Descriptive characteristics of physical development and functional capacity of the Lithuanian athletes in differents sports according to *ACTN3* C/T genotypes in ANOVA analysis is presented in Table 5. There was a significant difference in anaerobic index based on the genotype: STEMP in speed and power oriented sports of *ACTN3* T/T genotype athletes was higher compared to the STEMP of *ACTN3* C/C ir C/T genotype athletes in all other sport categories ($p<0.05$).

Table 4. Distribution of *ACTN3* C/T genotypes and alleles in the Lithuanian athletes and controls

Individuals	*N*	*Allele frequencies, %*		*ACTN3 gene C/T polymorphism genotype frequences, %*						χ^2	*p value*
		[C]	*[T]*	*[C][C]*		*[C][T]*		*[T][T]*			
				O	*E*	*O*	*E*	*O*	*E*		
Group I	101	60.4	39.6	29.7	35.5	61.4	47.8	8.9	15.7	8.1	0.0044
Group II	51	60.8	39.2	37.3	39.9	47.1	47.7	15.7	15.4	0.01	0.920
Group III	94	60.1	39.9	29.8	36.1	60.6	48.0	9.6	15.9	6.57	0.0103
Total athletes	246	60.4	39.6	31.3	36.0	58.1	47.8	10.6	15.7	11.35	0.0007
Lithuanian population	250	64.4	35.6	39.2	41.5	50.4	45.8	10.4	12.7	2.46	0.117

N – sample size. Group I – endurance sports representatives; group II – speed and power developing athletes; group III – representatives of game and duel sports; O – observed; E – expected (according to Hardy-Weinberg law, χ^2 and p).

Linear regression analysis showed that in athletes the *ACTN3* C/C and C/T genotypes, muscle mass and fat mass have a positive effect on AAMP ($\overline{R}^2$=0.419). The *ACTN3* C/T genotype, age, gender, mucle mass and sports category have a significant effect on STEMP ($\overline{R}^2$=0.524). It means that the older the athletes developing speed and power the better their resultant STEMP. There is positive relation between the women carrying *ACTN3* T/T ir C/T genotypes and speed-power characteristics. There are similar studies showing that in women of *ACTN3* T/T genotype the anaerobic muscle capacity was higher than in the carriers of *ACTN3* C/T ir C/C genotypes (Delmonico et al., 2007; Clarkson et al., 2005).

The Roufier index measuring aerobic capacity was lower in elite athletes of *ACTN3* C/T ir T/T genotype compared to RI of other athletes showing good training. According to statistical analysis the RI depends on age, fat mass and sport specifics. Athletes of *ACTN3* T/T genotype are more related to endurance demanding sports, however, heterozygotes, especially men, are related to endurance and speed-power demanding sports. We can conclude that *ACTN*3 T/T genotype is related to an increase of endurance.

Table 5. Descriptive characteristics of physical development and functional capacity of Lithuanian athletes in differents sports according to *ACTN3* C/T genotypes in ANOVA

Phenotypic index	*ACTN3 C/C genotype athletes*			*ACTN3 C/T genotype athletes*			*ACTN3 T/T genotype athletes*		
	Group I	***Group II***	***Group III***	***Group I***	***Group II***	***Group III***	***Group I***	***Group II***	***Group III***
Height, cm	180.4±8.9	178.0±9.8	176.7±8.4	182.2±8.4*	178.0±9.3*	173.5±8.8*	183.4±7.6	180.2±10.9	173.9±10.1
Weight, kg	71.2±10.3*	81.6±17.1*	66.6±9.5*	77.3±13.0*	80.3±16.9*	63.9±10.1*	79.5±12.6*	82.4±13.7*	61.6±10.3*
BMI, kg/m^2	21.7±1.7*	25.1±3.7*	21.8±2.0*	23.2±2.5*	25.4±4.3*	21.0±2.5*	23.1±2.4*	25.6±4.7*	20.3±2.0*
FM, kg	7.4±1.8*	10.2±5.7*	7.8±2.1*	8.1±2.4	8.7±4.3	8.2±3.1	8.5±1.5	9.3±3.2	8.1±3.5
MM, kg	38.7±6.8*	44.6±9.5*	34.7±5.9*	41.7±9.3*	44.7±9.1*	33.5±6.0*	43.9±7.5*	44.6±8.3*	31.1±6.3*
MFMI	5.5±1.7	4.9±1.4	4.6±0.8	5.6±1.5*	6.0±2.4*	4.5±1.2*	5.3±0.9	5.6±3.2	4.2±1.0
LV, l	5.5±0.7*	4.8±1.1*	4.4±0.7*	5.7±1.0*	4.7±1.0*	4.4±0.9*	5.7±0.6*	4.8±1.2*	4.2±0.9*
RHS, kg	46.9±10.1*	50.6±9.7*	41.4±6.8*	48.1±9.9*	52.8±9.4*	39.4±9.3*	48.6±10.7*	52.5±6.8*	36.8±7.8*
LHS, kg	47.0±8.6*	50.1±9.6*	40.9±8.9*	46.7±9.3*	51.3±9.7*	37.5±9.8*	48.2±11.2*	51.5±7.4*	35.4±9.4*
STEMP, W	1833.7±488.3	1913.3±529.9	1625.4±472.7	1727.8±492.7*	1987.7±441.1*	1510.7±418.4*	1810.7±463.5*	2215.4±575.6*	1474.3±357.5*
AAMP, W	1196.6±301.4	1228.4±290.9	1076.2±217.7	1180.7±336.8*	1220.5±204.1*	991.1±233.5*	1397.4±472.3*	1317.0±227.2*	986.3±155.8*
Roufier index	4.4±3.1	5.6±3.2	4.8±2.8	3.1±2.3*	6.0±2.8*	4.8±2.7*	2.0±1.5*	4.9±3.1*	5.8±4.1*
VO_2max, ml/min/kg	55.5±9.7	61.1±11.4	54.6±12.2	55.4±9.0	57.2±10.3	53.4±11.7	53.0±11.3	57.9±10.9	55.9±10.7

The data are presented as means ± standard deviations. * $p<0.05$ – significant differences between phenotypic indexes among sport groups according to genotype; group I – representatives of endurance sports; group II – representatives of speed and power demanding sports; group III – representatives of game sports and duels; BMI – body mass index; MFMI – mussle fat mass index; LV – life lung volume; STEMP – short term explosive muscle power; AAMP – anaerobic alactic muscle power; VO_2max – maximum oxygen consumption; FM- fat mass, MM – muscle mass; LHS – left handgrip strength; RHS – right handgrip strength.

Although there is an opinion that the deficit of *ACTN3* in fast twitch muscle fibers can reduce the human anaerobic capacity (Moran et al., 2007; Roth et al., 2007; Vincent et al., 2007; Ahmetov and Rogozkin, 2009), the athletes of *ACTN3* T/T genotype without functional α-actinin-3 protein in our study have good endurance, speed and power characteristics. Possibly, a deficit of α-actinin-3 is compensated by protein α-actinin-2 and/or other enzymes involved in anaerobic muscle activity.

In summary, the athletes of *ACTN3* C/T and T/T genotypes can achieve better indexes of muscle capacity in tasks which require maximum short-term efforts than C/C genotype athletes. In the Lithuanian athletes the *ACTN3* allele C is not a determinant of speed and power but rather has an additive effect on endurance.

Several laboratories are using *ACTN3* C/T polymorphism in genetic testing of young sportsmen and children in advising a choice of proper sport career. Therefore, assessment of sporting potential would be more informative taking into consideration a combination of several genes in Lithuania as well.

Analysis of *ACE* I/D and *AGTR1* A/C polymorphisms: results and discussion

Adaptation of cardiovascular system towards physical load is one among the main factors determining the general organism adaptation to environmental conditions. Renin-angiotensin system (RAS) plays a major role in regulation of blood suply and is responsible for internal homeostasis of the organism (Di Mauro et al., 2010; Jones and Woods, 2003). We have chosen the candidate genes and their markers, coding main components of RAS for our study: the first strong candidate determined by numerous investigations in different populations is *ACE* and its polymorphism (I/D); the second potential gene candidate is *AGTR1* and its polymorphism (C/T) which is responsible for the regulation of circulation.

Angiotensin-converting enzyme (ACE) is the most important component of RAS responsible for the formation of a strong vazoconstritor angiotensin II. ACE is present in many tissues and has a broad spectrum of activity. ACE breaks the proteins by removing their C-end amino acid sequences by converting angiotensin I into angiotensin II and

degrading the bradykinin. Angiotensin II which is formed through intermediate products is a strong vazoconstrictor. There are two receptors of angiotensin II: AGTR1 and AGTR2. Physiological function of angiotensin II is expressed through AGTR1 which is found in blood vessels, myocardium, skeletal muscle, kidneys and in tissues of sympatic nervous system. The receptors of AGTR2 start dominating in pathophysiological conditions (Abdollahi et al., 2005; Di Mauro et al., 2010).

According to literature and genomic databases, a mutation in the intron 16 of *ACE* gene leads to two allelic variants: (D) which is 287 bp length lacking the *Alu* sequence and (I) which possess this DNA fragment (Thompson et al., 2006; Nazarov et al., 2001; Ohno et al., 2005; Tanriverdi et al., 2005). In caucasians the *ACE* genotypes are distributed as follows: I/I – 25%, I/D – 50%, D/D – 25%, respectively. Since the mutation has been found in intron, it was assumed that it does not affect the functions of the enzyme. However, it was shown that these allelic variants have direct influence on the amount of ACE in blood serum: in the organisms of individuals carrying *ACE* D/D genotype the amount of ACE protein is two-fold higher compared to those of *ACE* I/I genotype (Amir et al., 2007; Lippi et al., 2009). According to literature, in endurance demanding sports the *ACE* I/I genotype is more frequent, wheras *ACE* D/D genotype was associated with speed and power demanding sports (Thompson et al., 2006; Nazarov et al., 2001; Ahmetov and Rogozkin, 2009; Amir et al., 2007), however, other studies refuted these associations (Mooren and Völker, 2005; Costa et al., 2009; Lippi et al., 2009).

Genotype and allele frequencies in Lithuanian athletes and general population are presented in Table 6. In our study the distribution of *ACE* I/D genotype frequencies in control group deviates from HWE, although the controls were sampled from geographically different ethnolinguistic groups. The genotype frequencies were significantly different between men and women in control group: the *ACE* D/D genotype was dominant in men, while heterozygous *ACE* I/D genotype prevailed in women. There was no deviation from HWE in the group of athletes (Table 6). In athletes the *ACE* D/D genotype and *ACE* D allele accordingly, was less frequent than in general population. In contrast to other studies (Baudin, 2002; Ohno et al., 2005), we found *ACE* D allele and D/D genotype more frequent in endurance demanding sports rather than in speed and

power-oriented one. Our data is in accordance with the results of the studies reporting the association of *ACE* D allele to endurance (Scott et al., 2005; Amir et al., 2007).

Table 6. Distribution of genotype and allele frequencies of *ACE* I/D polymorphism in Lithuanian atletes and controls

Individuals	*N*	*Allele frequencies, %*		*ACE gene I/D polymorphism genotype frequencies, %*						χ^2	*p value*
		[I]	*[D]*	*[I][I]*		*[I][D]*		*[D][D]*			
				O	*E*	*O*	*E*	*O*	*E*		
Group I	101	50.0	50.0	26.7	25.0	46.5	50.0	26.7	25.0	0.49	0.484
Group II	51	52.0	48.0	23.5*	27.0	56.9*	49.9	19.6*	23.1	0.99	0.32
Group III	478	48.7	51.3	25.3*	23.7	46.9*	50.0	27.8*	26.3	1.85	0.174
Total athletes	630	49.2*	50.8*	25.4*	24.2	47.6*	50.0	27.0*	25.8	1.41	0.235
Lithuanian population	250	42.6*	57.4*	23.6*	18.1	38.0*	48.9	38.4*	32.9	12.43	0.0004

N – sample size. Group I – endurance sports representatives; group II – speed and power developing athletes; group III – representatives of game and duel sports; O – observed; E – expected (according to Hardy-Weinberg law, χ^2 and p); * $p<0.05$ – significant differences in genotype and allele frequencies between athletes and controls.

Analysis of variance (ANOVA) revealed that in *ACE* I/I and I/D genotype athletes the body mass index, fat mass and muscle mass were significantly higher compared to *ACE* D/D genotype athletes ($p<0.05$). The *ACE* I/I genotype athletes in speed and power-oriented sports had higher STEMP and AAMP compared to those of *ACE* I/D and D/D genotype ($p<0.05$) (Table 7). Our study confirmed the findings of other groups showing that individuals of *ACE* I/I have higher indexes of anaerobic capacity than *ACE* I/D and D/D genotype individuals (Pescatello et al., 2006; Moran et al., 2006; Williams et al., 2005).

The endurance developing athletes of *ACE* D/D genotype have lower RI than *ACE* I/I and *ACE* I/D genotype (Table 7). It shows good training and aerobic capacity of the *ACE* D/D athletes. Regression analysis revealed that *ACE* I/I genotype, sport category, gender and age of an athlete and muscle mass of 52% determine the STEMP values ($\overline{R}^2$=0.52). There was statistically significant relationship between AAMP index and *ACE* I/I and I/D genotypes, gender and muscle mass ($\overline{R}^2$=0.443). It means that STEMP and AAMP are higher for athletes who have bigger muscle mass and lower fat mass.

We determined that in the athletes studied the *ACE* D/D genotype is associated with endurance, while *ACE* I/I genotype - with speed and power. The *ACE* I/D genotype athletes are in a middle position, they are related to both endurance and speed and power sports.

Table 7. Descriptive characteristics of physical development and functional capacity of Lithuanian athletes in differents sports according to *ACE* I/D genotypes in ANOVA

Phenotypic index	*ACE I/I genotype athletes*			*ACE I/D genotype athletes*			*ACE D/D genotype athletes*		
	Group I	***Ggroup II***	***Group III***	***Group I***	***Group II***	***Group III***	***Group I***	***Group II***	***Group III***
Height, cm	181.4±6.0	179.3±10.0	176.0±7.9	182.5±9.5*	178.3±8.2*	175.1±7.6*	181.4±8.9*	177.2±13.5*	171.9±11.3*
Weight, kg	76.0±8.9*	81.9±17.8*	65.2±8.9*	77.8±15.2*	82.5±15.5*	66.0±9.9*	73.6±11.3*	76.2±17.4*	60.6±10.6*
BMI, kg/m^2	22.9±2.4*	25.1±3.2*	21.1±1.9*	23.2±2.4*	25.9±4.3*	21.5±2.3*	22.1±2.1*	23.9±4.4*	19.9±2.4*
FM, kg	7.7±1.6	9.5±4.3	8.2±3.6	8.7±2.6	9.7±5.1	8.3±3.0	7.1±1.5	8.3±4.5	7.4±1.7
MM, kg	41.8±5.9*	45.6±9.8*	34.5±4.8*	42.5±8.8*	45.6±8.2*	34.8±5.8*	39.0±10.0*	40.8±9.9*	30.3±6.7*
MFMI	5.5±1.2	5.3±1.4	4.6±1.3	5.2±1.7	5.4±2.2	4.5±1.1	5.9±1.5*	6.0±3.1*	4.2±0.8*
LV, l	5.7±0.7*	5.1±1.1*	4.4±0.8*	5.6±0.7*	4.7±1.0*	4.5±0.8*	5.7±1.2*	4.5±1.2*	4.2±0.9*
RHS, kg	50.4±10.3*	52.0±10.5*	39.5±7.7*	48.0±10.8*	53.5±8.6*	40.2±8.7*	45.5±8.1*	47.5±7.7*	38.7±9.1*
LHS, kg	50.1±8.4*	50.5±11.0*	37.9±9.5*	46.5±9.8*	52.7±8.5*	38.9±9.9*	44.7±8.7*	46.2±7.5*	37.5±9.4*
STEMP, W	1902.8±465.8*	2103.4±594.5*	1585.8±395.0*	1801.4±498.9*	2013.4±453.2*	1553.2±376.9*	1592.4±449.8*	1815.2±500.3*	1473.9±544.0*
AAMP, W	1261.9±277.8*	1376.0±340.5*	998.6±234.1*	1231.1±381.5*	1179.2±182.3*	1048.9±194.7*	1127.4±346.9*	1245.8±201.9*	975.6±253.0*
Roufier index	4.2±2.6	5.5±3.7	5.5±3.1	3.2±2.8*	5.7±2.9*	4.8±3.1*	2.7±2.0*	5.8±2.4*	4.5±2.7*
VO_2max, ml/min/kg	51.5±8.4	57.6±13.3	56.0±10.2	57.9±9.8*	60.5±9.3*	53.4±11.2*	54.3±8.5	56.2±11.6	53.6±13.7

The data are presented as means ± standard deviations. * $p<0.05$ – significant differences between phenotypic indexes among sport groups according to genotype; group I – representatives of endurance sports; group II – representatives of speed and power demanding sports; group III – representatives of game sports and duels; BMI – body mass index; MFMI – mussle fat mass index; LV – life lung volume; STEMP – short term explosive muscle power; AAMP – anaerobic alactic muscle power; VO_2max – maximum oxygen consumption; FM- fat mass, MM – muscle mass; LHS – left handgrip strength; RHS – right handgrip strength.

Although *AGTR1* A/C (c.1166A>C) (rs5186) polymorphism is in 3'UTR, it can influence stability of iRNR and transcription. It is suggested that *AGTR1* C allele influences gene expression through the interaction with miRNR, resulting in increased *AGTR1* expression and angiothensin II influence (Plat et al., 2009; Di Mauro et al., 2010). The *AGTR1* A/C is assumed to relate to changes in cardiovascular system and impact on human anaerobic capacity (Jones and Woods, 2003; Plat et al., 2009; Di Mauro et al., 2010). Since angiothensin II regulates oxygen consuption and participates in muscle energy homeostasis and its physiological function is mediated by AGTR1, besides, the *AGTR1* A/C change impacts higher amount of angiothensin II in the blood serum, we hyphotesize that *AGTR1* C allele is related to human endurance and aerobic capacity.

We have studied 149 Lithuanian athletes and 240 individuals from general population. Genotype and allele frequencies are presented in Table 8.

Table 8. Distribution of genotype and allele frequencies of *AGTR1* A/C polymorphism in Lithuanian atletes and controls

Individuals	*N*	*Allele frequencies, %*		*AGTR1 gene A/C polymorphism genotype frequences, %*						χ^2	*p value*
		[A]	*[C]*	*[A][A]*		*[A][C]*		*[C][C]*			
				O	*E*	*O*	*E*	*O*	*E*		
Group I	77	70.8	29.2	50.6	50.1	40.3	41.4	9.1	8.5	0.05	0.823
Group II	30	66.7	33.3	40.0*	44.5	53.3*	44.5	6.7*	11.1	1.2	0.273
Group III	42	72.6	27.4	59.5	52.7	26.2	39.8	14.3	7.5	4.9	0.027
Total athletes	149	70.5	29.5	51.0	49.7	38.9	41.6	10.1	8.7	0.62	0.431
Lithuanian population	240	72.3	27.7	55.0*	52.3	34.6*	40.1	10.4*	7.7	4.49	0.034

N – sample size. Group I – endurance sports representatives; group II – speed and power developing athletes; group III – representatives of game and duel sports; O – observed; E – expected (according to Hardy-Weinberg law, χ^2 and p); * $p<0.05$ – significant differences in genotype and allele frequencies between athletes and controls.

Distribution of *AGTR1* A/C genotypes in the control group deviates from HWE, although the controls represent geographically distinct ethnolinguistic groups. In athletes the genotype distribution agrees with HWE, except in the group of game and duel sports (Table 8). According to NCBI data, in Asian population frequency of the rare *AGTR1* C allele is 3–4% but in European populations it varies from 25% to 34.8% (dbNCBI). In Lithuanian population the frequency of *AGTR1* C allele is 27.7%, while in the athlete group it is 29.5%. In speed and power group the *AGTR1* C allele was more frequent

(33.3%) compared to other sport groups and controls ($p>0.05$). There were no females in this sport group carrying *AGTR1* C/C genotype, also in all the group *AGTR1* A/C heterozygous genotype was dominant.

Analysis of variance revealed the significant differences between men and women according to *AGTR1* A/C polymorphism. The height, weight, muscle mass, MFMI, LV, handgrip strength and indexes of functional capacity STEMP, AAMP, RI, and VO_2max were significantly different ($p<0.05$). We found that men of genotype *AGTR1* A/A had higher VO_2max which characterizes the function of the vascular and respiratory systems compared to the men of the *AGTR1* A/C and C/C genotypes ($p<0.05$). The statistical differences of the phenotypic indexes between the sport groups according to *AGTR1* A/C polymorphism are presented in Table 9.

Regression analysis in all the athletes showed that gender, fat mass and muscle mass are strongly related to AAMP ($\overline{R}^2$=0.575). The high muscle mass and low fat mass of an athlete leads to improved AAMP. In speed and power sports, the men, carriers of *AGTR1* A/A genotype, and women, carriers of the *AGTR1* A/C genotype, have higher AAMP. The infuence of *AGTR1* A/C polymorphism on the physical capacity phenotype is different depending on gender.

There was relationship between STEMP and muscle mass (positive) and *AGTR1* A/C genotype ($\overline{R}^2$=0.421). High STEMP was observed in speed and power-oriented sports in men of *AGTR1* A/A genotype and women of *AGTR1* A/C genotype, and in edurance oriented sports, in men of C/C genotype.

Table 9. Descriptive characteristics of physical development and functional capacity of Lithuanian athletes in differents sports according to *AGTR1* A/C genotypes in ANOVA

Phenotypic index	*AGTR1 A/A genotype athletes*			*AGTR1 A/C genotype athletes*			*AGTR1 C/C genotype athletes*		
	Group I	***Group II***	***Group III***	***Group I***	***Group II***	***Group III***	***Group I***	***Group II***	***Group III***
Height , cm	182.2±8.90*	183.5±8.79*	176.3±6.8*	180.4±8.1	180.1±9.3	176.6±9.1	186.93±5.0*	192.0±8.5*	171.2±7.6*
Weight , kg	75.6±14.3*	86.7±16.8*	67.4±8.8*	75.0±10.2*	84.3±11.8*	66.9±9.3*	82.9±10.3*	88.5±7.8*	59.2±6.5*
BMI, kg/m^2	22.5±2.6*	25.9±4.8*	21.7±2.3*	23.0±2.0*	25.4±3.3*	21.4±1.8*	23.2±2.3*	23.9±0.3*	20.3±1.2*
FM, kg	7.9±2.3	10.5±7.0	8.9±3.3	7.9±2.1	9.4±4.2	8.2±1.8	7.6±1.2	8.1±0.6	6.3±0.8
MM, kg	41.2±8.5*	47.3±9.0*	35.7±5.1*	40.3±9.1*	46.4±7.3*	35.2±4.5*	45.4±5.7*	46.9±9.9*	31.2±3.3*
MFMI	5.4±1.6*	5.6±2.4*	4.4±1.1*	5.5±1.6	5.9±3.2	4.6±1.1	5.9±0.6	5.8±0.8	4.9±1.0
LV, l	5.6±0.8*	5.2±0.8*	4.7±0.7*	5.8±0.9*	5.2±1.0*	4.6±0.6*	6.2±0.5*	6.2±0.8*	4.3±0.9*
RHS, kg	46.9±8.8	50.2±9.4§	40.7±7.1	47.7±10.4	55.3±9.4§	40.7±10.7	54.0±12.4	62.0±11.3§	39.0±11.4
LHS, kg	45.9±9.2	49.5±10.1	40.1±8.3	47.2±9.1	54.3±9.0	39.1±11.9	51.8±9.9*	53.5±16.3*	36.2±10.7*
STEMP, W	1760.4±521.6*	2134.9±491.5*	1675.4±340.4*	1742.7±459.18*	2149.6±519.2*	1789.6±401.9*	1877.0±427.3*	2328.0±135.7*	1319.8±317.9*
AAMP, W	1214.4±265.9	1262.5±243.3	1102.5±191.1	1168.2±316.4	1314.0±281.3	1041.0±245.6	1378.7±224.0*	1432.0±151.3*	992.6±157.4*
Roufie index	3.5±2.2*	5.8±3.5*§	5.7±2.9*	3.4±3.1*	6.0±3.5*§	4.5±2.8*	2.1±1.4*	8.2±3.1*§	5.2±1.0*
VO_2max, ml/min/kg	57.5±9.8§	56.3±10.5§	57.9±11.7§	53.6±8.8§	54.3±14.3§	54.7±12.7§	49.6±7.7§	47.8±6.8§	47.3±6.8§

The data are presented as means ± standard deviations. * $p<0.05$ – significant differences in phenotypic indexes betwee sport groups according to genotype; $^{\S}p<0.05$ – significant phenotypic differences between *AGTR1* (A/A, A/C, C/C) genotypes; group I – representatives of endurance sports; group II – representatives of speed and power demanding sports; group III – representatives of game sports and duels; BMI – body mass index; MFMI – mussle fat mass index; LV – life lung volume; STEMP – short term explosive muscle power; AAMP – anaerobic alactic muscle power; VO_2max – maximum oxygen consumption; FM- fat mass, MM – muscle mass; LHS – left handgrip strength; RHS – right handgrip strength.

The Roufie index depends on age, fat mass, sport group and *AGTR1* C/C genotype ($\overline{R}^2$=0.220). VO_2max index of athletes of *AGTR1* A/A genotype is higher than of those carrying *AGTR1* A/C and C/C genotypes ($p<0.05$). Summarizing the results of regression anlysis we conclude that in the all athletes the *AGTR1* (A/A, A/C and C/C) genotypes show unique characteristics and they can be related to both speed and power and endurance.

Currently active research efforts are directed towards individual components of the RAS system aiming at elucidation of their specific influences onto stuctural and morphological changes in cardio vascular system (Di Mauro et al., 2010). It was shown that the *ACE* D/D and *AGTR1* C/C genotypes are related to the increased risk of cardiovasular diseases (stroke, ventricle hypertrophy, and hypertension) (Ashley et al., 2006; Baudin, 2002). However, systematically administered physical load increases working heart capacity. Genomic investigation/testing would facilitate the athlete training and would allow administering optimal physical load in surveilance and prevention of chronic diseases. It also would help to establish strategies of personalized physical training.

Our study revealed that *ACE* I/D polymorphism is strongly associated with phenotypes of physical fitness of Lithuanian athletes (I/I genotype is related to phenotypes optimal in speed and power, and D/D genotype to those of endurance), however, we were not able to elucidate any strong association of *AGTR1* A/C polymorphism with phenotypes of physical fitness.

Analysis of associations of *PPAR* family regulator gene markers: results and discussion

Human adaptation to physical strain depends on the activity of many genes. Regulatory genes *PPARGC1A*, *PPARA*, *PPARG,* participating in energy and lipid metabolism, play an important role in this process of adaptation. We investigated the *PPARGC1A* G/A (rs8192678), *PPARA* G/C (rs4253778), *PPARG* C/G (rs1801282) polymorphisms and their influence upon physical development and functional capacity in professional Lithuanian athletes.

According to literature and genomic databases, peroksisome-proliferator activated receptor (*PPAR*) gene family belongs to the group of regulatory genes, coding nuclear receptors PPARα, PPARδ ir PPARγ (dbNCBI; dbNRR; Baar, 2004). PPAR receptors act as transcription factors and regulate expression of many genes. A composition of muscle fibers and metabolism in muscle tissue depend on the activity of PPAR receptors. Each *PPAR* is activated by specific ligand, especially in stress (for example, in starvation or high physical load) (Liang and Walter, 2006; Lippi et al., 2009).

PPARa regulates energy homeostasis, glucose and lipid circulation, it controls body mass and constriction of blood vessels (Lefebvre et al., 2006). The PPARa is more active in slow twitching endurance muscle fibers than in the fast twitching ones (Russel et al., 2003; Ahmetov and Rogozkin, 2009). Oxidative properties of fast twitching muscle depend on the activity of *PPARA* gene products (Russel et al., 2003; Lefebvre et al., 2006; Flavell et al., 2005). It is accumed that PPARa is important for adaptation of the organism to various levels of physical strain. Recently, there is interest in G/C polymorphism in intron 7 of *PPARA* gene (Flavell et al., 2005; Ahmetov et al., 2006; Ahmetov et al., 2009; Lippi et al., 2009; Bray et al., 2009). Despite the polymorphism being in intronic region it seems to have an important function. It may cause lower *PPARA* gene expression (Ahmetov et al., 2006). *PPARA* C allele influences heart hypertrophy and is related to reduced fat acid oxidation (FAO) and *PPARA* expression (Flavell et al., 2005; Lefebvre et al., 2006; Liang and Walter, 2006). If *PPARA* G/C polymorphism influences FAO and glucose metabolism in the heart and skeletal muscle, then the single nucleotide change may be related to phenotypes of human physical capacity. It was hypothesized that *PPARA* G allele may influence human endurance, and C allele is related to improved human speed and power features (Baar, 2004; Ahmetov et al., 2006; Lippi et al., 2009).

Frequency of *PPARA* C allele in different populations is different: in Europe it is 19%, while in Afroamericans it is 62.5%, however, in Asia there was no indication of *PPARA* C allele (dbNCBI). According to our data, in Lithuanian population the frequency of *PPARA* C allele is 17.2%, and in athletes it is 21.1%. The frequencies of genotypes and alleles in Lithuanian athletes and controls are presented in Table 10.

Table 10. Distribution of genotype and allele frequencies of *PPARA* G/C polymorphism in Lithuanian athletes and controls

Individuals	*N*	*Allele frequencies, %*		*PPARA G/C polymorphism genotype frequences, %*						χ^2	*p value*
		[G]	*[C]*	*[G][G]*		*[G][C]*		*[C][C]*			
				O	*E*	*O*	*E*	*O*	*E*		
Group I	101	74.8*	25.2*	58.4*	55.9	32.7*	37.7	8.9*	6.4	1.82	0.177
Group II	51	75.5	24.5	54.9	57.0	41.2	37.0	3.9	6.0	0.65	0.420
Group III	228	81.6	18.4	68.4	66.5	26.3	30.1	5.3	3.4	3.53	0.060
Total athletes	380	78.9	21.1	63.9	62.3	30.0	33.2	6.1	4.4	3.61	0.057
Lithuanian population	250	82.8*	17.2*	69.2*	68.6	27.2*	28.5	3.6*	3.1	0.51	0.475

N – sample size. Group I – endurance sports representatives; group II – speed and power developing athletes; group III – representatives of game and duel sports; O – observed; E – expected (according to Hardy-Weinberg law, χ^2 and p); * $p<0.05$ – significant differences in genotype and allele frequencies between athletes and controls.

There were no deviations from Hardy-Weinberg equilibrium of *PPARA* G/C genotype distribution neither in athletes nor in controls ($p>0.05$). The allele frequencies in male athletes (*PPARA* G/C: 79.0/21.0) differed significantly from those in men of general Lithuanian population (*PPARA* G/C: 84.7/15.3) ($p=0.03$). The difference was noted comparing the groups of men athletes developing speed power and endurance against the men in the control groups. In endurance sports the frequency of *PPARA* C allele (25.2%) was significantly higher compared to the control group (17.2%, $p=0.015$). The allele frequencies in skiers (*PPARA* G/C: 66.7/33.3) and in kayak/canoe athletes (*PPARA* G/C: 63.5/36.5) were significantly different from the controls (*PPARA* G/C: 82.8/17.2) ($p<0.05$). There were no females of C/C genotype in the group of game and duel sports. The *PPARA* G/C and C/C genotypes were significantly more frequent among the athletes in speed, power and endurance sports than G/G genotype compared to the individuals in the control group ($p<0.05$). Our study showed that occurrence of *PPARA* C allele increases with athletic qualification: among elite athletes it was (30.2%) much more frequent than in other athletes (sub-elite – 21.4%; non-elite – 19.5%) and controls (17.2%) ($p>0.05$). A case-control study performed by Russian scientists in 2006 showed the opposite results: they found that *PPARA* C allele was rather rare in endurance athletes (11%) compared to speed and power athletes (27%) and control group (16%) (Ahmetov et al., 2006). Apparently the human physical capacity in different populations may be determined by different genomic factors.

In ANOVA we observed that indexes of physical developmen (height, weight, muscle mass, MFMI, LV, handgrip strength) and indexes of functional capacity (AAMP, RI) were different between men and women ($p<0.05$). These differences arise due to physiological differences between the genders; however, genotypic influence is also important. We found that height and muscle mass in men, carriers of the *PPARA* G/C genotype, were significantly higher than in carriers of the *PPARA* G/G and C/C genotypes ($p<0.05$). The differences of the phenotypic indexes between the sport groups according to *PPARA* G/C polymorphism are presented in Table 11.

Linear regression analysis revealed that STEMP of speed-power athletes is significantly affected by *PPARA* C/C and G/C genotypes and muscle mass ($\overline{R}^2$=0.548), while AAMP is more affected by *PPARA* C/C genotype, muscle mass and fat mass ($\overline{R}^2$=0.408). The effects of *PPARA* G/C polymorphism on phenotypes are different in different genders. In summary, the athletes of *PPARA* C/C ir G/C genotype have improved speed and power features, and *PPARA* G/G genotype athletes have improved endurance features.

We partially confirmed the hypothesis that *PPARA* G allele influence on human endurance and *PPARA* C allele is related to the speed and power (Baar, 2004; Ahmetov et al., 2006; Lippi et al., 2009).

Another member of PPAR, PPARγ, is essential for differentiation of mioblasts and adipots. The PPARγ is important in adipogenesis and lipid methabolism (Liang and Walter, 2006; Ehrenborg and Krook, 2009; Ruchat et al., 2010). A C/G polymorphism in *PPARG* gene 2 (B) exon (c.34C>G, p.Pro12Ala,) was identified, influencing the effieciency of the *PPARG* gene transcription and relating to insulinn metabolism (Eriksson et al., 2003; Ehrenborg and Krook, 2009; Ruchat et al., 2010). Similarly to *PPARA* G/C, the *PPARG* C/G polymorphism and environmental factors affect various aspects of metabolism during physical load (Eriksson et al., 2003; Lippi et al., 2009).

Table 11. Descriptive characteristics of physical development and functional capacity of Lithuanian athletes in differents sports according to *PPARA* G/C genotypes in ANOVA

Phenotypic index	*PPARA G/G genotype athletes*			*PPARA G/C genotype athletes*			*PPARA C/C genotype athletes*		
	Group I	***Group II***	***GroupIII***	***Ggroup I***	***Group II***	***Group III***	***Group I***	***Group II***	***Group III***
Height, cm	179.8±8.1§	177.5±9.5	175.2±9.3	185.0±8.1*§	179.1±9.8*	172.8±8.2*	184.6±7.8§	181.0±14.1	176.3±1.5
Weight, kg	73.0±10.3*§	81.1±15.5*	63.6±9.6*	80.8±14.2*§	81.9±17.7*	65.9±11.5*	78.5±16.4§	73.2±16.6	66.5±3.3
BMI, kg/m^2	22.5±2.1*	25.5±3.8*	20.6±2.1*	23.3±2.6*	25.5±4.6*	21.6±2.8*	23.0±3.1	22.4±1.7	21.4±0.9
FM, kg	8.0±2.6*	10.2±5.2*	7.4±2.2*§	8.0±2.1	8.5±4.1	9.0±3.5§	7.1±2.0*	6.1±1.3*	11.8±3.6*§
MM, kg	39.3±8.2*	43.7±8.5*	32.8±5.8*	44.1±8.3*	46.2±9.7*	34.9±6.9*	43.4±10.3	43.0±10.2	35.4±2.9
MFMI	5.4±1.6	4.8±1.3§	4.7±0.9§	5.7±1.5*	6.3±1.5*§	4.2±1.1*§	6.1±0.5*	7.4±3.3*§	3.1±0.8*§
LV, l	5.6±0.9*	4.7±1.1*	4.2±0.8*	5.8±0.9*	4.8±1.0*	4.4±0.9*	5.4±0.8	4.5±2.2	4.0±0.1
RHS, kg	46.9±9.9*	51.1±8.1*	40.2±8.1*	48.9±10.3*	53.7±10.3*	39.3±9.3*	50.8±9.2*	46.0±8.5*	33.0±4.3*
LHS, kg	45.8±9.5*	50.5±8.8*	39.2±9.3*	48.3±8.1*	52.1±9.8*	36.9±10.5*	50.2±11.7	44.0±5.6	33.0±6.1
STEMP, W	1736.5±436.5*	1928.7±503.0*	1480.9±427.0*	1752.3±511.1*	2088.7±509.5*	1644.0±433.1*	2022.3±718.5	1958.0±176.8	1749.3±209.0
AAMP, W	1186.8±301.4*	1228.6±222.6*	1002.9±211.1*	1228.1±352.0	1251.7±274.0	1054.1±256.9	1277.3±400.6	1240.0±237.6	974.7±47.1
Roufier index	5.5±3.0*	4.9±2.9*	4.5±2.9*	3.6±2.2*	5.9±3.1*	4.4±2.9*	1.8±2.4*	4.8±0.0*	8.3±1.6*
VO_2max, ml/min/kg	55.2±9.2	57.6±11.8	53.2±11.2	55.3±10.2	60.9±8.9	57.1±13.2	54.5±6.9	62.7±0.0	49.6±0.9

The data are presented as means ± standard deviations. * $p<0.05$ – significant differences in phenotypic indexes betwee sport groups according to genotype; $^{\S}p<0.05$ – significant phenotypic differences between *PPARA* (G/G, G/C, C/C) genotypes; group I – representatives of endurance sports; group II – representatives of speed and power demanding sports; group III – representatives of game sports and duels; BMI – body mass index; MFMI – mussle fat mass index; LV – life lung volume; STEMP – short term explosive muscle power; AAMP – anaerobic alactic muscle power; VO_2max – maximum oxygen consumption; FM- fat mass, MM – muscle mass; LHS – left handgrip strength; RHS – right handgrip strength.

It is known that *PPARG* G allele is related to decreased activity of PPARγ, increasing the insulin sensitivity and consumption of glucose (Eriksson et al., 2003; Ehrenborg and Krook, 2009; Ruchat et al., 2010). Influence of *PPARG* upon the adaptation of the organism to physical load has been little investigated.

It was hypothesized that *PPARG* G allele is related to human speed and power because of decreased transcriptional activity of PPARγ which causes lower aerobic endurance in humans, however, the *PPARG* C allele is related to aerobic endurance (Ahmetov et al., 2008; Lippi et al., 2009; Ruchat et al., 2010).

Frequency of the rare *PPARG* G allele differs in populations: in European populations it reaches 15–20%, in Afroamericans it is 12%**,** while in Asian populations is up to 1% (dbNCBI). In our study there was no deviation from HWE either in Lithuanian athletes or in general population. Genotype and allele frequencies are presented in Table 12. In general Lithuanian population the frequency of *PPARG* G allele was 16.4%, in athletes it was 12.8%, thus, there was no statistically significant difference observed ($p>0.05$).

We observed statistically significant differences in genotype and allele frequences neither between sport groups nor between the groups of different athletic qualification.

In general Lithuanian population the *PPARG* G/G genotype is observed in men (3%), and in women (2.4%), however, there were only a few athletes carrying G/G genotype (1.2%) (one male in speed power sports and two females in the endurance sports). This constrained our statistical analysis of phenotypes to only *PPARG* C/C and C/G genotype groups. ANOVA detected significant differences in anthropometric indexes. In speed and power sports athletes had better palm strength and STEMP than in the endurance group. There were significant differences between genders. The males of *PPARG* C/C genotype had higher indexes of physical development such as height, weight, muscle mass, MFMI, LV, palm strength, anaerobic capacity AAMP and Rufie index, and less fat mass than women of *PPARG* C/C genotype ($p<0.05$); the MFMI, palm strength and AARG of men with *PPARG* C/G genotype were higher than those of women of *PPARG*

C/G genotype ($p<0.05$). These differences arise because of physiological differences between males and female, however, genetic influences can not be discarded.

Table 12. Distribution of genotype and allele frequencies of *PPARG* C/G polymorphism in Lithuanian athletes and controls

Individuals	*N*	*Allele frequencies, %*		*PPARG C/G polymorphism genotype frequences, %*						χ^2	*p value*
		[C]	*[G]*	*[C][C]*		*[C][G]*		*[G][G]*			
				O	*E*	*O*	*E*	*O*	*E*		
Group I	101	86.1	13.9	74.3	74.2	23.8	23.9	2.0	1.9	0.0	1.0
Group II	51	86.3	13.7	74.5	72.7	23.5	25.1	2.0	2.2	0.01	0.920
Group III	94	89.6	10.6	78.7	79.9	21.3	19.0	0.0	1.1	1.33	0.249
Total athletes	246	87.2	12.8	75.6	76.0	23.2	22.3	1.2	1.7	0.35	0.554
Lithuanian population	250	83.6	16.4	70.0	69.9	27.2	27.4	2.8	2.7	0.02	0.887

N – sample size. Group I – endurance sports representatives; group II – speed and power developing athletes; group III – representatives of game and duel sports; O – observed; E – expected (according to Hardy-Weinberg law, χ^2 and p).

Statistical differences of phenotypic indexes between different sport groups according to *PPARG* C/G polymorphism are presented in Table 13. In speed and power group the athletes of *PPARG* C/G genotype had significantly higher STEMP and AAMP than in other sport groups ($p<0.05$). In endurance group the *PPARG* C/C ir C/G genotype athletes had lower Roufier index than athletes of the same genotype in other sports ($p<0.05$). It shows good training and aerobic capacity of the athletes. In the elite group the sportsmen of *PPARG* C/C genotype had significantly higher height, weight and strength of both palms compared to the sportsmen with heterozygous *PPARG* C/G genotype (elite, sub-elite and non-elite) ($p<0.05$).

We hypothesized that in the presence of *PPARG* G allele (heterozygous C/G genotype), the anaerobic capacity of the athletes is increased, and, hence, the speed and power features dominate. In C/C genotype, when the G allele is absent, the features of aerobic endurance are more expressed.

Based on linear regression we found that STEMP (53%) variation is explained by the *PPARG* C/C and C/G genotypes, sport group, fat and muscle mass ($\overline{R}^2$=0.533). It means that high STEMP is characteristic of the athletes of *PPARG* C/G genotype in speed and power group compared to endurance group. The AAMP (59%) depends on *PPARG* C/C ir C/G genotype, gender, fat and muscle mass ($\overline{R}^2$=0.59). Linear regression analysis

showed that the athletes who have more muscles and less fat mass possess better STEMP and AAMP indexes.

We partially confirmed our hypothesis (also of others) that *PPARG* G allele influences human endurance, and *PPARG* C allele is related to speed and power (Ahmetov et al., 2008; Lippi et al., 2009).

Based on literature and information of genomic databases, the ligand activation of PPARα and PPARγ needs transcriptional coactivator which is peroxisome proliferator activated δ receptor coactivator 1α (coded by *PPARGC1A* gene). The *PPARGC1A* is a transcriptional coactivator of many nuclear receptors (PPARα, PPARδ, PPARγ, α estrogene and β estrogene receptors) (Baar, 2004; Liang and Walter, 2006). An expression of *PPARGC1A* takes place in muscle and fat cells. The *PPARGC1A* participates in the metabolism of carbonhydrates and lipids, in the increase of the amount of mitochondria, in a diferentiation of myoblasts and adipocytes, in thermogenesis (Baar, 2004; Lehman et al., 2008). The *PPARGC1A* gene expression significantly increases during the physical activity. The *PPARGC1A* participates in oxidative processes in heart myocytes, and in skeletal muscle it facilitates formation of slow muscle fibers (Russel et al., 2003; Stefan et al., 2007).

The *PPARGC1A* 8 exon G/A (c.1444G>A; p.Gly482Ser) polymorphism is related to FAO, functions of vascular and respiratory systems and affects insulin sensitivity in liver and skeletal muscle (Liang et al., 2006; Lehman et al., 2008; Nitz et al., 2007). According to literature the *PPARGC1* A allele is assumed to be a risk factor of type 2 diabetes, obesity and decreased oxygen consuption (Jamshidi et al., 2002; Lucia et al., 2005; Franks et al., 2003; Stefan et al., 2007). However, these results are controvesial.

Table 13. Descriptive characteristics of physical development and functional capacity of Lithuanian athletes in differents sports according to *PPARG* C/C and C/G genotypes in ANOVA

Phenotypic index	*PPARG C/C genotype athletes*			*PPARG C/G genotype athletes*		
	Group I	***Group II***	***Group III***	***Group I***	***Group II***	***Group III***
Height , cm	182.7±8.3*	178.8±8.9*	173.7±9.3*	180.0±7.6	177.8±11.5	177.0±6.7
Weight , kg	77.4±13.0*	81.7±15.7*	63.5±10.3*	72.4±10.3*	81.4±17.8*	67.1±8.6*
BMI, kg/m^2	22.9±2.4*	25.4±4.1*	20.8±2.3*	22.2±2.0*	25.5±4.1*	21.5±2.1*
FM, kg	8.1±2.3	9.4±5.2	7.9±2.8	7.4±1.6	9.4±2.8	8.3±3.2
MM, kg	42.3±7.7*§	45.1±8.3*	33.1±6.2*	39.9±6.5*§	44.2±10.6*	34.9±5.6*
MFMI	5.5±1.6*	5.7±2.5*	4.4±1.1*	5.4±1.4	4.9±1.1	4.7±1.0
LV, l	5.6±0.8*	4.8±1.0*	4.3±0.9*	5.7±1.0*	5.0±1.3*	4.5±0.6*
RHS, kg	48.8±9.9*	51.4±8.0*	39.2±8.3*	45.5±10.0*	54.4±11.9*	40.8±8.9*
LHS, kg	47.9±9.7*	50.5±7.2*	38.1±10.1*	44.3±7.4*	53.4±13.3*	38.7±8.2*
STEMP, W	1795.8±506.3*	1976.9±468.2*	1486.4±424.6*§	1690.2±420.2*	2123.0±554.4*	1708.0±397.1*§
AAMP, W	1227.7±372.7*	1225.9±218.9*	1006.5±217.5*	1157.3±254.4	1281.8±346.0	1047.6±232.8
Roufier index	3.4±2.7*	5.9±3.1*	5.2±2.9*	3.3±2.0	4.8±2.6	3.9±2.7
VO_2max, ml/min/kg	55.9±9.2*	59.7±11.1*	52.5±11.0*§	55.0±9.8	57.0±10.5	59.0±12.2§

The data are presented as means ± standard deviations. * $p<0.05$ – significant differences in phenotypic indexes betwee sport groups according to genotype; $^{\S}p<0.05$ – significant differences of phenotypic indexes according to *PPARG* C/C and G/C genotype groups; group I – representatives of endurance sports; group II – representatives of speed and power demanding sports; group III – representatives of game sports and duels; BMI – body mass index; MFMI – mussle fat mass index; LV – life lung volume; STEMP – short term explosive muscle power; AAMP – anaerobic alactic muscle power; VO_2max – maximum oxygen consumption; FM- fat mass, MM – muscle mass; LHS – left handgrip strength; RHS – right handgrip strength.

It is also suggestted that the *PPARGC1* G allele is associated with human endurance (Franks et al., 2003; Stefan et al., 2007; Ahmetov and Rogozkin, 2009; Lucia et al., 2005). Individuals of the *PPARGC1* G/G and G/A genotype have more intensive FAO in liver, miocardium, skeletal muscle compared to A/A genotype. It was hypothesized that the *PPARGC1* G allele influences human aerobic endurance, and the A allele – human speed and power (Franks et al., 2003; Stefan et al., 2007; Ahmetov and Rogozkin, 2009).

In our study there was no deviation from HWE of genotype distribution in Lithuanian athletes and controls ($p>0.05$). The genotype and allele frequencies are presented in Table 14.

Table 14. Distribution of genotype and allele frequencies of *PPARGC1A* G/A polymorphism in Lithuanian athletes and controls

Individuals	***N***	***Allele frequencies, %***		***PPARGC1A G/A polymorphism genotype frequences, %***						χ^2	***p value***
		[G]	***[A]***	***[G][G]***		***[G][A]***		***[A][A]***			
				O	***E***	***O***	***E***	***O***	***E***		
Group I	101	74.3	25.7	54.5	55.1	39.6	38.3	5.9	6.6	0.13	0.718
Group II	51	77.5	22.5	56.9	60.0	41.2	34.9	2.0	5.1	1.63	0.202
Group III	455	70.1	29.9	49.2	49.2	41.8	41.9	9.0	8.9	0.01	0.920
Total athletes	607	71.4	28.6	50.7	51.0	41.4	40.8	7.9	8.2	0.1	0.752
Lithuanian population	250	72.4	27.6	51.6	52.4	41.6	40.0	6.8	7.6	0.42	0.517

N – sample size. Group I – endurance sports representatives; group II – speed and power developing athletes; group III – representatives of game and duel sports; O – observed; E – expected (according to Hardy-Weinberg law, χ^2 and p).

The rare *PPARGC1* A allele in Lithuanian population had frequency of 27.6% (in other European populations it is from 30% to 43%), whereas among athletes it was 27.7%. The women athletes of *PPARGC1A* A/A genotype were found only among the athletes of games and duel sports (10.5%). There were no significant differences in allele and genotype frequencies among the investigated groups.

Only Lithuanian male athletes in endurance, speed and power sports had *PPARGC1A* A/A genotype. Analysis of phenotypic characteristics showed that only muscle fat mass index was higher in *PPARGC1A* A/A genotype male athletes compared to *PPARGC1A* G/A and G/G genotypes ($p=0.0008$). The indexes of anaerobic capacity STEMP and AAMP were higher in *PPARGC1A* A/A genotype sportsmen compared to those carrying

the *PPARGC1A* G/G and G/A genotypes ($p<0.05$). Analysis of aerobic capacity showed that maximum oxygen consumption (VO_2max) was better in *PPARGC1A* G/G genotype male athletes than in those of the G/A and A/A genotypes. Our data corroborates the findings of others that the athletes of *PPARGC1A* G/G genotype have higher VO_2max than *PPARGC1A* A/A genotype athletes (Franks et al., 2003; Lucia et al., 2005; Stefan et al., 2007).

Statistical differences of phenotypic indexes between different sport groups according to *PPARGC1A* G/A polymorphism are in Table 15. The athletes of endurance group had better RI compared to other athletes ($p<0.05$). The elite athletes of *PPARGC1A* G/G genotype had significanltly better indexes of physical development (height, weight, MM, MFMI, LV), and the RI reflecting the aerobic capacity was lower ($p<0.05$) compared to other athletes. These differences show possible genetic influences upon the development of the athlete and his/her characteristics of physical capacity.

Based on linear regression analysis there is a significant relationship of the age, muscle mass, *PPARGC1A* G/A genotypes and sport group of the athletes to the STEMP ($\overline{R}^2$=0.528). The AAMP depends positively on the muscle mass and the genotype (G/A ir A/A) of *PPARGC1A* G/A polymorphism ($\overline{R}^2$=0.408). We conclude that the athletes of *PPARGC1A* G/A and A/A genotypes are related more to speed and power sports, while *PPARGC1A* G/G genotype athletes relate more to endurance demanding sports. Hetrozygous *PPARGC1A* G/A athletes were in the middle, they related to both speed and power and endurance sports.

In summary, the *PPARGC1A* G/A polymorphism are associated with the phenotype of physical fitness in Lithuanian athletes: the *PPARGC1A* G/A and A/A genotypes are related to speed and power sports, and *PPARGC1A* G/G genotype, to endurance demanding sports. We confirmed the hypothesis that the *PPARGC1* G allele influences human aerobic endurance, while *PPARGC1* A allele is more related to improving speed and power features.

Table 15. Descriptive characteristics of physical development and functional capacity of Lithuanian athletes in different sports according to *PPARGC1A* G/A genotypes in ANOVA

Phenotypic index	***PPARGC1A G/G genotype athletes***			***PPARGC1A G/A genotype athletes***			***PPARGC1A A/A genotype athletes***		
	Group I	***Group II***	***Group III***	***Group I***	***Group II***	***Group III***	***Group I***	***Group II***	***Group III***
Height , cm	180.9±8.5*	178.6±10.2*	173.4±8.1*	182.6±8.6*	177.3±8.4*	175.0±9.2*	185.4±4.2	192.5±0.0	177.5±10.3
Weight , kg	75.5±13.2*	80.6±17.5*	62.7±9.2*	75.1±11.2*	81.9±15.2*	65.1±10.4*	87.5±5.8*	81.0±0.0*	68.7±10.9*
BMI, kg/m^2	22.9±2.6*	25.0±4.4*	20.8±2.3*	22.4±2.0*	26.1±3.7*	20.9±2.2*	24.8±1.6	21.8±0.0	21.7±2.8
FM, kg	7.8±2.6*	9.5±2.6*	7.3±2.2*§	8.3±1.6	9.5±3.5	8.4±3.0§	6.7±1.2	3.2±0.0	10.0±4.3§
MM, kg	41.4±8.1*	43.9±9.2*	32.9±5.8*	40.1±9.4*	45.6±9.4*	33.6±6.1*	47.7±2.9*	45.6±0.0*	35.8±7.1*
MFMI	5.6±1.7*§	5.5±2.3*§	4.7±0.9*	5.1±1.1*§	5.2±1.1*§	4.3±1.1*	7.3±1.5*§	14.2±0.0*§	3.9±1.2*
LV, l	5.6±0.9*	4.9±0.9*	4.3±0.8*	5.6±0.9*	4.5±1.0*	4.5±0.8*	6.2±0.5*	5.7±0.0*	4.4±1.1*
RHS, kg	46.7±9.7*§	51.9±10.0*	40.4±6.8*	47.7±9.6*§	51.3±7.2*	38.4±9.1*	61.0±6.0*§	68.0±0.0*	41.3±11.9*
LHS, kg	45.3±8.9*§	50.8±10.1*	38.0±8.5*	47.3±8.6*§	50.4±7.6*	38.0±10.4*	60.5±8.2*§	65.0±0.0*	40.7±11.3*
STEMP, W	1774.6±540.7*	1961.6±508.2*	1488.7±409.3*	1746.4±442.5*	1999.8±462.9*	1580.3±441.4*	1801.4±242.9*	2898.0±0.0*	1594.4±469.0*
AAMP, W	1144.0±301.4*	1246.4±223.9*	1022.4±213.9*	1249.1±327.8*	1211.8±262.2*	1012.8±229.*3	1496.7±142.1*	1572.0±0.0*	1008.7±240.9*
Roufier index	3.7±2.7*	5.6±2.9*	4.3±2.5*	3.0±2.5*	5.5±2.9*	5.6±2.9*	2.4±1.6	10.4±0.0	4.7±4.6
VO_2max, ml/min/kg	54.6±7.9	61.3±9.3	54.4±12.7	55.6±10.9	56.4±11.7	52.5±10.3	59.1±12.1	57.9±1.0	59.1±12.1

The data are presented as means ± standard deviations. * $p<0.05$ – significant differences in phenotypic indexes betwee sport groups according to genotype; § $p<0.05$ – significant phenotypic differences between *PPARGC1A* (G/G, G/A, A/A) genotypes; group I – representatives of endurance sports; group II – representatives of speed and power demanding sports; group III – representatives of game sports and duels; BMI – body mass index; MFMI – mussle fat mass index; LV – life lung volume; STEMP – short term explosive muscle power; AAMP – anaerobic alactic muscle power; VO_2max – maximum oxygen consumption; FM- fat mass, MM – muscle mass; LHS – left handgrip strength; RHS – right handgrip strength.

Analysis and discussion of the researched candidate gene variant combinations

Scientists in collaboration with trainers attempt to create the training program which would lead to the best sporting achievements. The long term main task of the trainer is to facilitate the dvelopment of the dominant individual features of an athlete. Genetic testing would allow trainers to prepare the most efficient training strategies. Adjusting the amounts of physical load during the training would enable the development of the features important for a specific sport maintaining physical integrity of an athlete (Ahmetov et al., 2009; Lippi et al., 2009), since training of the individual traits is important for every sport.

A combination of gene variants, strongly related to human physical performance, as well as their interaction, can help in determining the differences in the athletic capacity (Ahmetov et al., 2009, Lippi et al., 2009; Williams and Folland, 2008). Complex analysis of gene associations with physical fitness can be carried out in two ways. One of them is to determine the most frequent combination of genotypes in athletes different from the control group (Ahmetov et al., 2009; Lippi et al., 2009; Williams and Folland, 2008).

Based on the investigated *ACTN3* C/T, *AGTR1* A/C, *ACE* I/D, *PPARGC1A* G/A, *PPARA* G/C, *PPARG* C/G polymorphisms, in a specific sport group the Lithuanian athletes have characteristic combinations of the genotypes (determined from the genotype distributions and statistically significant associations with the phenotypes):

- In endurance sports –

ACTN3 [C/T] / *AGTR1* [A/C] / *ACE* [I/I] / *PPARGC1A* [G/G] / *PPARA* [C/C] or [G/C] / *PPARG* [C/C];

- In speed and power sports –

ACTN3 [T/T] / *AGTR1* [A/C] / *ACE* [I/D] / *PPARGC1A* [G/A] or [A/A] / *PPARA* [G/C] / *PPARG* [C/C];

- In game and duel sports –

ACTN3 [C/T] / *AGTR1* [C/C] / *ACE* [I/D] / *PPARGC1A* [A/A] / *PPARA* [C/C] / *PPARG* [C/C].

Another method is based on the evaluation of the alleles associated with physical performance. The alleles, which are statistically associated with the intermediate phenotypes determining the end point phenotype, are combined (Ahmetov et al., 2009). In our study certain combinations of the alleles occur in the specific sports:

- In endurance sports –

ACTN3 [T] / *AGTR1* [C] / *ACE* [D] / *PPARGC1A* [G] / *PPARA* [G] or [C] / *PPARG* [C];

- In speed and power sports –

ACTN3 [T] or [C] / *AGTR1* [C] / *ACE* [I] / *PPARGC1A* [A] / *PPARA* [C] / *PPARG* [G];

- In game and duel sports –

ACTN3 [T] / *AGTR1* [A] / *ACE* [I] or [D] / *PPARGC1A* [A] / *PPARA* [C] or [G] / *PPARG* [C].

The largest part of DNA samples in the biobase of the Lithuaninan athletes were taken from the representatives of games and duel sports (n=478), mostly football players (n=400). With the help of trainers, the sportsmen were divided into categories based on their positions in the field (forwards, defenders, halfbacks and goalkeapers). We estimated the *ACE* I/D, *PPARGC1A* G/A, *PPARA* G/C allele and genotype distributions in the above categories.

We found that according to the categories based on the field position the athletes have following characteristic allele combinations:

- forwards – *ACE* [I] or [D] / *PPARGC1A* [A] / *PPARA* [C];
- defenders – *ACE* [I] / *PPARGC1A* [A] / *PPARA* [C];
- halfbacks – *ACE* [I] / *PPARGC1A* [G] / *PPARA* [G] or [C];
- goalkeapers – *ACE* [I] / *PPARGC1A* [G] / *PPARA* [G].

Sport scientists state that athletes practicing in game sports require interaction of both aerobic and anaerobic sytems and *status quo* of their regulatory mechanisms. We have confirmed this opinion.

A. G. Williams ir J. P. Folland in 2008 suggested a way of evaluation of the most suitable, best combination of genotypes for improved endurance. Their model allows to compute a probability that a person will have a specific genotype combination according to the polymorphisms selected and their frequencies in population based on *total genotype score* (TGS). The TGS value changes from 0 (an individual lacks improved endurance features) to a 100 (an athlete with excellent endurance features). The model of Williams and Folland (W–F) proposes a way of quantitative characterization of impact of genotype combinations upon a specific phenotype.

Based on W–F method, we evaluated by TGS the combination of the genotypes which are best suited for endurance in the Lithuanian athletes and controls. We used the genotypes of our investigated genetic variants (*ACE* I/D, *ACTN3* C/T, *PPARGC1A* G/A, *PPARA* G/C and *PPARG* C/G). In Lithuanian population the mean TGS value was 66.36±13.2, wheras in athletes it was 65.6±13.8. The TGS values were distributed between 30 and 100. A single athlete had an "ideal" genotypic combination for endurance (football player). The 8.3% of the athletes and the 4.3% of the controls had TGS close to 90. The percentages of the athletes who had TGS in the interval from 70 to 100 were distributed as follows: in endurance group 22.3%, in speed-power group 15.0%, and in games and duel sports 16.6%.

We performed more extensive analysis of TGS, since there was no significant difference between the athletes and controls. The TGS was computed for all the athletes according to their endurance phenotype (for endurance and games-duel sports because these athletes have developed aerobic and anaerobic capacity). The average TGS in the total group was 65.7±13.9, and in subgroups it was not much different from the total group: in elite group 65.4±13.8; in sub-elite group 64.9±15.0; in non-elite group 66.4±13.2.

In order to test whether this complex phenotype of physical fitness in inherited in our population we looked for significant difference of TGS in the Lithuanian athletes and general population. If the athletes do not differ from the controls in endurance, there might be a detectable difference in genotype combination optimal for speed and power. We united the athletes characterized by improved speed and power, including the ones

from games and duel sports, into one group. We used the genotypes of the investigated genetic variants *ACE* I/I, *ACTN3* C/C, *PPARGC1A* A/A, *PPARA* C/C ir *PPARG* G/G. Based on this subdivision in general Lithuanian population the average TGS value was 33.64±13.2, and in the united group of the athletes it was 34.6±13.9. However, the elite athletes had TGS of 44.4±11.3, which is larger than that of sub-elite 36.7±16.2, non-elite 32.6±13.2 and controls 33.64±13.2. Although total TGS in speed and power group was smaller than that in the endurance group, there was a detectable difference between the athletes and controls. The TGS values in athletes and controls were distributed between 0 and 70, the mean value being 30. The TGS value of 50 was observed in 15.5% of the athletes, but only 10.5% in the general population.

We confirmed our hypothesis which is also accepted in the area of sports genetics that speed and power features tend to be more inherited than acquired in training. However, the features of improved endurance can be developed and strengtened during long-term training (Beunen and Thomis, 2006; Calvo et al., 2002; Lippi et al., 2009).

By assessing the importance of genotype combinations to the inheritance of the multifactorial trait it is possible to estimate a probability of how many individuals from the given general population will have an "ideal" genetic profile with respect to that trait. We found that the probability of an individual possesing an ideal endurance genetic profile according to the five investigated polymorphisms is 1%, and the probability of optimal speed and power profile is 0.0007%. Hence, each 99th Lithuanian can possess an optimal genotype combination for endurance, and only every 132 650th can have the combination optimal for speed and power. Optimal genotype combination can be observed more frequently only in very large population. The more polymorphisms are included in the analysis, the lower are chances to find the optimal genotype combination (Ahmetov et al., 2009; Williams, Folland, 2008).

CONCLUSIONS

1. By using modern bioinformatics methods, the following candidate genes and their polymorphisms were selected:

- *ACTN3 (*α-actinin-3 gene) c.1747C>T (p.R577X, rs1815739);
- *ACE* (angiotensin converting enzyme gene) I/D (*Alu* sequence insertion/deletion);
- *AGTR1* (angiotensin II type 1 receptor) c.1166A>C, (rs5186);
- *PPARGC1A* (peroxysome proliferator activated δ receptor coactivator 1α gene) c.1444G>A, (p.Gly482Ser, rs8192678);
- *PPARA* (peroxysome proliferator activated receptor gene) c.2528G>C (rs4253778);
- *PPARG* (peroxysome proliferator activated γ receptor gene) c.34C>G, (p.Pro12Ala, rs1801282).

2. The frequency distributions of the alleles/genotypes of the candidate gene markers in the Lithuanian athletes and general population had a specific pattern. Typical genotype/allele combinations were observed in different sport groups:

- *ACTN3* c.1747C>T polymorphism T/T genotype athletes adapt well to physical strain and achieve high results in any sport discipline.
- *ACE* I/D polymorphism D/D genotype Lithuanian athletes have typically higher endurance, I/I genotype athletes, speed and power.
- Athletes with any *AGTR1* c.1166A>C polymorphism genotype have characteristically higher endurance as well as speed and power.
- *PPARGC1A* c.1444G>A polymorphism G/G genotype and *PPARG* c.34C>G polymorphism C/C genotype athletes have typical long-term adaptation to endurance requiring physical strain.

3. The indexes of physical development and functional capacity in the Lithuanian athletes attain the levels of elite. Statistical analysis of phenotypic indexes revealed the inherited qualities of the athletes and how they adapt to physical load. The genotypes

investigated have different influence on physical capacity of males and females; they are statistically significantly associated with the indexes of physical capacity:

- The athletes, carriers of the *ACTN3* T/T, *ACE* I/I, *PPARGC1A* A/A, *PPARA* C/C genotypes, typically have better ability to achieve high muscle capacity indexes when exercising short-term explosive muscle power tasks.
- The athletes, carriers of the *ACTN3* T/T and C/T, *AGTR1* A/A, *ACE* D/D, *PPARGC1A* G/G, *PPARA* G/G, *PPARG* C/C and C/G genotypes, typically have better cardiovascular system indexes which are associated with aerobic capacity.

4. The genetic diversity of physical capacity in the Lithuanian population has the pattern similar to that of other populations.

- *ACTN3* T/T genotype athletes without the functional protein ACTN3 in the fast-twitch muscle fibers possess the properties of aerobic and anaerobic capacity. We confirmed the opinion of other researchers that lack of ACTN3 is compensated. Although some laboratories recommend the testing of young athletes according to the *ACTN3* C/T polymorphism, we conclude that in the Lithuanian population the use of a combination of genes is more appropriate.
- The results of our research contradict the work published by other researchers stating that *ACE* D/D genotype is more frequent in athletes compared to the general population, but corroborate the results of the researchers who determined that the D allele was associated with endurance.
- We did not confirm the hypothesis that *AGTR1* C allele is associated with human endurance and aerobic capacity but found that the C/C genotype is typical in athletes from game and duel sports.
- We confirm the hypothesis of other researchers who claim *PPARA* G and *PPARGC1* G alleles to have influence on human aerobic endurance, while the *PPARA* C and *PPARGC1* A alleles are associated with speed and power qualities. Due to sample size limitations the hypothesis that *PPARG* G allele influences endurance was only partially confirmed.

- Our results agree with the opinion of many sport specialists, practitioners and genetic researchers that speed and power qualities are more inherited than acquired compared to the endurance qualities.

5. We created the DNA biobase of Lithuanian elite athletes of various sports disciplines and collected information concerning the genotypes and phenotypes of physical development and functional capacity of the athletes.

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SANTRAUKA

Žmonių populiacijų tyrimai atskleidžia jų fizinio pajėgumo genetinę įvairovę. Tokių ypatumų turi ir Lietuvos populiacija. Sportininkų individualaus genomo žinojimas ypač svarbus sporto teorijai, praktikai ir medicinai. Darbas skirtas svarbiausiems klausimams, susijusiems su genetinių veiksnių įtaka sportinio fizinio pajėgumo komponentams. Darbo metu buvo sukaupta Lietuvos didelio meistriškumo sportininkų imtis, kuri ištirta genetiškai ir pagal fenotipą. Sukurtoje Lietuvos didelio meistriškumo įvairių sporto šakų sportininkų DNR mėginių biobazėje sukaupta informacija apie sportininkų genotipus ir sportininkų fizinio išsivystymo bei funkcinio pajėgumo fenotipiniai duomenys. Parinkti stipriausi genai kandidatai, siejami su žmogaus fiziniu pajėgumu. Didelio meistriškumo sportininkai pirmą kartą Lietuvoje buvo tirti pagal 6 genų kandidatų DNR žymenų alelių, dažniausiai asocijuojamų su fiziniu pajėgumu, paplitimą: *ACTN3 (*α-aktinino-3 geno) c.1747C>T (p.R577X, rs1815739), *ACE* (angiotenziną konvertuojančio fermento geno) I/D (*Alu* sekos insercija/delecija); *AGTR1* (pirmo tipo angiotenzino II receptoriaus) c.1166A>C, (rs5186), *PPARGC1A* (peroksisomų proliferatoriaus aktyvinto δ receptoriaus koaktyvatoriaus 1α geno) c.1444G>A, (p.Gly482Ser, rs8192678); *PPARA* (peroksisomų proliferatoriaus aktyvinto α receptoriaus geno) c.2528G>C (rs4253778); *PPARG* (peroksisomų proliferatoriaus aktyvinto γ receptoriaus geno) c.34C>G, (p.Pro12Ala, rs1801282)

Tirtų genų kandidatų žymenų genotipų/alelių dažnių įvairovė išskirtose sportininkų grupėse ir bendroje Lietuvos populiacijoje turi savitumų. Visų tirtų Lietuvos sportininkų fizinio išsivystymo ir funkcinio pajėgumo rodikliai atitinka didelio meistriškumo sportininkų lygį. Fenotipinių rodiklių statistinė analizė parodė sportininkų organizmo įgimtus gebėjimus ir prisitaikymą prie fizinių krūvių. Kiekvienos išskirtos sporto šakų grupės sportininkams būdinga genotipų/alelių kombinacija.

ACTN3 T/T genotipo Lietuvos sportininkai gerai prisitaiko prie fizinių krūvių ir pasiekia gerų sportinių rezultatų bet kurioje sporto šakoje. *ACE* D/D genotipo sportininkams būdinga ištvermė, I/I genotipo – greitis ir jėga. *AGTR1* A/C polimorfizmo visų genotipų Lietuvos sportininkams būdinga tiek greitis ir jėga, tiek ir ištvermė. *PPARGC1A* G/G

genotipo ir *PPARG* C/C genotipo sportininkams būdinga ištvermė, o *PPARA* C/C genotipo – greitis ir jėga.

Tirtų genetinių variantų genotipai turi skirtingos įtakos vyrų bei moterų fiziniam pajėgumui ir yra statistiškai reikšmingai asocijuoti su sportininko fizinio pajėgumo rodikliais. Darbo metu nustatyta, kad paveldimumas turi didesnę reikšmę žmogaus greičio ir jėgos savybėms nei ištvermės.

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2. **Ginevičienė V**, Kučinskas V, Kasnauskienė J, Pranckevičienė E, Milašius K. The effect of *ACE* and *PGC1A* genetic variants on the physical capacity of elite Lithuanian athletes. 2nd Baltic States sport science conference. *Scientific management of high performance athletes' coaching.* Vilnius, Lithuania, April 23–25, 2009.

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