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Relating fitness phenotypes to genotypes in Lithuanian elite athletes

Valentina Ginevičienė[1],

Erinija Pranckevičienė[2],

Kazys Milašius[3],

Vaidutis Kučinskas[1]

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

[2] Department of Biology, University of Ottawa, Canada

[3] Vilnius Pedagogical University, Lithuania

Background. We aim to link fitness-related genotypes to the development of specific physical fitness phenotypes and a predisposition towards a specific sport category in Lithuanian elite athletes.

Materials and methods. The study involved 193 athletes (152 male and 41 female) and 250 controls. The athletes were stratified into four groups: endurance, mixed sports, speed / power, and team sports. Genotypes of the athletes were identified according to the genetic polymorphisms: *ACE* (rs1799752), *ACTN3* (rs1815739), *PPARGC1A* (rs8192678) and *PPARA* (rs4253778). One-way analysis of variance and logistic regression modelling were used for testing the genotype–phenotype association.

Results. The frequency of *ACE* I allele was higher in athletes than in controls, although only male athletes showed a significant difference from male controls. The *PPARA* C allele was more common in the athlete group than in the general population of Lithuania. There were no significant *ACTN3* and *PPARGC1A* SNP allele / genotype frequency differences between the athlete group and the controls. We have found that the *ACTN3* RR genotype is associated with single muscular contraction power; the *PPARGC1A* Ser482Ser was associated with the muscle fat mass index; the *PPARA* CC and *ACE* II genotypes are associated with the muscle mass and single muscular contraction power. The effect of the gene variants was different for male and female athletes.

Conclusions. The *ACE* II, *PPARA* CC, *ACTN3* RR genotypes are related to the speed/power sports and the *ACE* DD, *PPARA* GG are related to the endurance sports in Lithuanian athletes. *PPARGC1A* Ser482Ser may be not critical but rather additive to endurance performance.

Key words: physical performance, *ACE*, *ACTN3*, *PPARGC1A* and *PPARA* genetic variants, sport category

INTRODUCTION

Physical performance is a quantitative multi-factorial inherited trait in which a phenotypic expression is influenced by both multiple genes and environmental factors. In recent years, many studies have attempted to find the candidate genes that influence human physical performance (1). The phenotypes of physical performance with a suspected genetic basis include endurance capacity, muscle performance, ability of tendons and ligaments to resist the effect of injury, and physiological attitude towards training. They also depend on the athletes' sex and the type of sport practiced. Since physical fitness has a strong genetic component, athletes might be predisposed towards increased performance in a specific sport discipline, which can be pivotal for the advancement of their career in sport (1–3).

Genetic variants. The disparities in human physical performance can be explained by differences in genetic makeup and environmental variation (3, 4). Our study was limited to four common polymorphisms selected because of previously reported associations with various aspects of metabolism: *ACE* (NCBI ref. SNP ID: rs1799752) insertion / deletion I/D polymorphism in a short intronic *Alu* sequence (transposon repeat polymorphism) and single nucleotide polymorphisms

Correspondence 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

(SNPs) in *ACTN3* R/X (NCBI ref. SNP ID: rs1815739), *PPARGC1A* Gly482Ser (NCBI ref. SNP ID: rs8192678), and *PPARA* G/C (NCBI ref. SNP ID: rs4253778).

The human angiotensin-1-coverting enzyme (*ACE*) gene was one of the first genes to be associated with human physical performance. The protein encoded by the *ACE* gene is the most important component of the rennin–angiotensin system (3–5). The *ACE* gene is located on chromosome 17 (17q22-q24). The polymorphism in intron 16 of *ACE* is described by the presence (insertion) or absence (deletion) of a 287-base-pair *Alu* sequence within the *ACE* gene. Reports on the effects of I/D polymorphism vary across studies and populations. In endurance sports, the II genotype (homozygous for insert I) is more frequent, whereas the DD genotype (homozygous for deletion D) dominates in speed / power sports (5–8). These associations have been refuted by other researchers (9–11). Recent studies in Lithuania have shown that the frequency of the *ACE* D allele is lower in the athlete group as compared to the group from general population; also, the DD genotype is more frequent in the endurance sports group compared to the speed / power group (12).

α-Actinin 3 (*ACTN3*) is one of the three human genes coding for proteins called α-actinins which are important in binding and anchoring actin filaments in human muscles. The α-actinins play a key role in the maintenance and regulation of the cytoskeleton (3, 13). The *ACTN3* gene is located on chromosome 11 (11q13-q14); a common polymorphism identified in humans results in a premature stop codon and lack of detectable protein. This C>T transversion at position 1747 in exon 16 converts an arginine codon into a stop codon, resulting in a protein with only 577 amino acids (p.Arg577X) (14, 15). It is suggested that athletes with the *ACTN3* XX genotype are more likely to compete in endurance sports and those with the *ACTN3* RR genotype in speed / power sports (3, 8, 13, 15, 16).

The peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1 α) is a transcriptional factor which impacts multiple aspects of cellular energy metabolism, including mitochondrial biogenesis and cellular respiration, the regulation of adaptive thermogenesis, adipocyte cell development, and lipid and glucose metabolism (13, 17–19). The *PPARGC1A* gene is located on chromosome 4 (4p15.1). A common coding single nucleotide polymorphism (c.1444G>A) in exon eight causes an amino acid substitution Gly482Ser (13, 19, 20). It is hypothesized that the Gly482 allele of *PPARGC1A* is associated with endurance (8, 21). The 482Ser allele is associated with a slower metabolism (18). People with the *PPARGC1A* Ser482Ser genotype are suspected to have a reduced aerobic efficiency (8, 13, 18, 21, 22).

The peroxisome proliferator-activated receptor α (PPARα) is a nuclear receptor that regulates liver and skeletal muscle lipid metabolism as well as glucose homeostasis (17, 18, 22). Endurance training increases the use of non-plasma fatty acids and may enhance skeletal muscle oxidative capacity through *PPARA* regulation of gene expression (22). The *PPARA* gene is located on chromosome 22 (22q12-q13.1), and a (G/C) SNP (c.2528G>C) was discovered in the intron 7 of the *PPARA* gene (13, 23). It is hypothesized that individuals with the *PPARA* GG and GC genotypes have increased rates of fatty acid oxidation in the liver, myocardium and in skeletal muscle cells (13, 24). Individuals with the *PPARA* CC genotype tend to have an increased anaerobic metabolism (13, 24). The *PPARA* G allele is suspected to associate with endurance and the C allele with speed / power (8, 24).

Genetic testing might be useful for the development of genetic performance tests; they may also be applied for pre-participation risk screening and may therefore prevent sudden deaths during sport. In the future, genetic testing might also be used to identify those who are most likely to benefit medically from certain exercise programs and may become more important in anti-doping (anti-gene doping) activities as with time the risk of the use of gene therapy to increase physical performance will increase; therefore, genetic testing could be used for direct anti-doping testing (2).

Physical fitness phenotypes. In sports medicine, phenotypes of physical fitness are standardized. Athletes excelling in a specific sport (endurance or speed / power), are characterized by a specific fitness phenotype (25). Several measures of physical fitness, reflecting overall physical and cardiovascular capacity, are used in the assessment of athletes. These measures comprise fat mass (FM), muscle mass (MM), body mass index (BMI), muscle fat mass index (MFMI), single muscu-

Table 1. **Optimal sport-specific fitness phenotypes of Lithuanian elite athletes (Skernevičius et al., 2004)**

Physical fitness measures	Endurance sports		Mix sports		Speed / power sports		Team sports	
	Males	Females	Males	Females	Males	Females	Males	Females
MM	42.02 (C)	28.3–31.1	48.6 (CK/R)	34.2	–	–	41.5–49	35.1–35.5
FM	6.8 (C)	10.9	8.1 (CK/R)	–	–	–	7.6–9.0	10.5–12.9
BMI	22.6 (C)	24.1 (C)	25.9 (CK/R)	–	–	–	21.3–23.4	–
MFMI	6.4 (C)	3.7–4.2	6.34 (CK/R)	–	4.6 (W)	4.02	4.97	2.9–3.9
SMCP	24.9–28.3	19.7–21.1	26.6–29.4	20.3	33.3	27.8	25.7	22.1
AAMP	13.7–15.7	11.8–13.7	17.6	14.7	17.6–21.9	15.7–19.6	16.6	15.3
RI	1.0–(–2.0)	4.9	0–4.0	4.0–6.0	2.0–5.0	3.0–6.0	1.6–3.2	2.0–4.0

FM, fat mass (kg); MM, muscle mass (kg), BMI, body mass index (kg/m^2); MFMI, muscle fat mass index; SMCP, single muscular contraction power (W/kg); AAMP, anaerobic alactic muscular power (W/kg); RI, Roufier index. (C), cycling; (CK/R) canoe-kayak rowing; (W), wrestlers.

lar contraction power (SMCP), anaerobic alactic muscular power (AAMP) and the Roufier index (RI). Optimal values of physical fitness for different sport disciplines for males and females are summarized in Table 1 as the guidelines for the assessment of Lithuanian athletes (25, 26).

Although a great number of genes and genetic markers have been already reported, showing the association with physical performance phenotypes and health-related good fitness, our aim is to provide the reader with the knowledge about the importance of *ACE*, *ACTN3*, *PPARGC1A* and *PPARA* genetic variants to phenotypes associated with physical performance in Lithuanian elite athletes. To our knowledge, this is the first population-based study in Lithuania that has observed an interaction between the four polymorphisms and physical activity. The present study has two goals. The first goal is a comparison of the *ACE* I/D, *ACTN3* R/X, *PPARGC1A* Gly482Ser and *PPARA* G/C genotype and allele frequencies between the athletes and the controls, and the second goal is a linking of the genotypes of these genetic polymorphisms to physical performance and sports category in Lithuanian athletes.

MATERIALS AND METHODS

Subjects. In total, 193 male (n = 152) and female (n = 41) Lithuanian athletes (age 22.0 ± 6.3 years) of regional or national competitive standard were recruited from the following sports: biathlon (n = 5), cross-country skiing (n = 12), road cycling (n = 12), pentathlon (n = 4), swimming (n = 13), rowing (n = 9), track and field (long distance) athletics (n = 9), track and field (short distance) athletics (n = 20), kayaking (n = 13), weightlifting (n = 31), boxing (n = 6), wrestling (n = 10), tennis (n = 3), soccer (n = 32) and handball (n = 14). The athletes were practicing in the four different sports categories: endurance (n = 64), speed / power (n = 47), mix (n = 33)) and team (n = 49) sports. We restricted our analysis to the elite athletes only, since they represented a homogeneous group with respect to the acquired training and physical fitness. Controls were 250 healthy unrelated citizens (167 males and 83 females; 36.2 ± 7.2 years) from all ethnoliguistic groups of Lithuanians (East, West and South Aukstaiciai and North, South and West Zemaiciai) without any competitive sport experience. The athletes and control groups were all Caucasians. The Lithuanian Bioethics Committee approved the study, and written informed consent was obtained from each participant.

Physical fitness phenotypes. Physical fitness phenotypes were measured using height (cm), body mass (kg), fat mass (kg), muscle mass (kg), body mass index (kg/m^2), muscle–fat mass index. Evaluation of single muscular contraction power (kgm/s/kg) (SMCP, vertical jump test) was based on the Bosco methodology (27), and anaerobic alactic muscular power (AAMP, stair-climb test) (kgm/s/kg) was estimated by using the Margaria test (28). Pulmonary and cardiovascular system capacities were assessed by the Roufier index (RI). The RI is a functional test consisting of a combined evaluation of heart rate at rest, during reaction to physical strain and during recovery after physical strain (29).

Genotyping. Genomic DNA was extracted from peripheral blood leukocytes by the phenol–chloroform extraction technique. The *ACE* specific region was amplified by polymerase chain reaction (PCR). PCR amplicons of 190 bp fragment for the D allele and 477 bp fragment for the I allele were separated on 2% agarose gel by electrophoresis and visualized by ethidium bromide staining as described previously (30). Genotyping for the *ACTN3* R/X polymorphism was carried out using the *DdeI* restriction enzyme described by Mills and colleagues (31). In brief, digested PCR fragments (108 bp, 97 bp, 86 bp fragments for X allele and 205 bp, 86 bp for R allele) were separated by 8% polyacrylamide gel electrophoresis and stained with ethidium bromide (31). Genotyping for the *PPARA* G/C variant was performed by PCR and restriction enzyme digestion (32). PCR products were incubated with the restriction enzyme *Taq1* which cleaved the amplicon (266 bp) from the C allele into two fragments 216 bp and 50 bp in length. The products of digestion were then separated by 2% agarose gel electrophoresis (32). The analyzed PCR product of the *PPARGC1A* Gly482Ser polymorphism was cut by *MspI* restriction endonuclease. Digested PCR fragments (449 bp fragment for 482Ser allele and 274 bp, 175 bp for Gly482 allele) were separated by 2% agarose gel and stained with ethidium bromide (33).

Statistical analysis. The genotype frequencies of the athletes were tested for compatibility with Hardy–Weinberg equilibrium (HWE). A chi-square (χ^2) test was used to assess the fit of the observed genotype frequencies with the HWE. One-way analysis of variance (ANOVA) was used to test for significant differences in the fitness phenotypes among the genotypes. The association of multivariate fitness phenotypes with individual genotypes was inferred by logistic regression (LR) modelling with backward variable selection, adjusted for the different sports categories (34–36):

$$\frac{P(g = G \mid x_1, x_2, \ldots, x_n)}{1 - P(g = G \mid x_1, x_2, \ldots, x_n)} = \\ = \exp\left(b_0 + b_1 x_1 + b_2 x_2 + \ldots + b_n x_n\right). \quad [1]$$

In Equation (1), the expression $P(g = G \mid x_1, x_2, \ldots, x_n)$ denotes the probability of the genotype G, given the independent variables x_i, $i = 1, \ldots, n$ which represent the investigated fitness-related phenotypic variables ($n = 7$). The right-hand-side formula in equation (1) computes the odds in favour of the genotype G given the actual values of the fitness variables. An impact of a change of the variable x_i by one unit, while other variables are fixed, on an increase / decrease of the odds is measured by $\exp(b_i)$, called the odds ratio (OR). The odds ratio is a quantitative measure of the significance of the phenotypic variable in predicting the specific genotype. An adjustment for a possible confounding by the sports group was done by including a variable coding for the sports category

Table 2. **Allele and genotype frequencies of four genetic variants in Lithuanian athletes and controls**

Sports groups	N	*ACE* genotype frequencies, %			Allele frequencies		*ACTN3* genotype frequencies, %			Allele frequencies		*PPARGC1A* genotype frequencies, %			Allele frequencies		*PPARA* genotype frequencies, %			Allele frequencies	
		II	ID	DD	I	D	RR	RX	XX	R	X	GlyGly	GlySer	SerSer	Gly	Ser	GG	GC	CC	G	C
Group I (endurance)	64	21.9	45.3	32.8	0.445	0.555	26.6	62.5	10.9	0.578	0.422	50.0	46.9	3.1	0.734	0.266	60.9	34.4	4.7	0.781	0.219
Group II (speed / power)	47	21.3	59.6	19.1	0.511	0.489	34.0	53.2	12.8	0.606	0.394	48.9[†]	48.9	2.2	0.734	0.266	53.2	44.7	2.1	0.755	0.245
Group III (mix)	33	42.4[§]	36.4	21.2	0.606	0.394	33.3	54.5	12.2	0.606	0.394	66.7	24.2	9.1	0.788	0.212	54.5	33.3	12.2	0.712	0.288
Group IV (team sports)	49	24.5	46.9	28.6	0480	0.503	30.6	32.6	16.3	0.571	0.429	42.9	44.9	12.2	0.653	0.347	71.4	22.5	6.1	0.827	0.173
Total	193	25.9*	47.7	26.4	0.497	0.503	30.6	56.5	12.9	0.588	0.412	50.8	43.0	6.2	0.723	0.277	60.6	33.7	5.7	0.775	0.225
Controls	250	23.6	38	38.4	0.426	0.574	39.2	50.4	10.4	0.644	0.356	51.6	41.6	6.8	0.724	0.276	69.2	27.2	3.6	0.828	0.172

*$\chi^2 = 7.35$, df = 2, p = 0.025 for ACE genotype frequencies in total athletes versus control subjects; [§] $\chi^2 = 6.39$; df = 2; p = 0.041 for ACE genotype frequencies in 'mix' athletes versus control subjects; [†] $\chi^2 = 6.01$; df = 2; p = 0.049 for PPARGC1 genotype frequencies in 'speed / power' athletes versus 'mix' athletes.

in the full LR model. Variables remaining in the LR model at the $p < 0.05$ significance level after backward variable selection were assumed significantly related to the genotype.

Our rationale for using the Logistic Regression modelling in inferring the genotype / allele relationship to the phenotypic variables is the following. Assuming that allele A exerts a strong influence on the fitness phenotype modelled by a continuous variable F, we expect that the OR associated with the variable F will be significant in predicting a homozygous AA or a heterozygous Aa genotype. Large values of the variable F associate with either the AA or Aa genotypes if the corresponding OR is greater than 1. If the OR is inferior to 1, then small values of F are associated with the AA or Aa genotypes. Trends in the values of the identified significant phenotypic variables that associated with the specific genotypes were matched to the phenotypes dominating in the different sports categories (endurance, speed / power, mix and team, see Tables 3 and 1). The observed correspondences suggest the possible genotype / allele associations with the endurance, speed / power and other sports categories. Statistical analysis was performed with SPSS (version 13.0). Male and female athletes were analyzed separately.

RESULTS

The distribution of polymorphisms in 193 Lithuanian athletes and in 250 healthy untrained control individuals is shown in Table 2. The *ACE* genotype Hardy–Weinberg equilibrium (HWE) calculations showed no deviation from expected frequencies in the total athlete group ($\chi^2 = 0.42$; df = 1; p = 0.517), but a deviation was observed in controls ($\chi^2 = 12.43$; df = 1; p = 0.0004) (Table 2). In the control group, significant differences in genotype frequencies were determined between males and females (II/ID/DD: 24.6/29.9/45.5% vs. 21.7/54.2/24.1%; $\chi^2 = 15.4$; df = 2; p = 0.0005). However, after the grouping of the athletes according to sports groups and sex, significant differences were determined in the allele frequencies of the *ACE* gene between the total athlete group and the control group (I/D: 49.7/50.3%, vs. 42.6/57.4; $\chi^2 = 4.48$; df = 1; p = 0.034); male athletes showed differences from the control group (I/D: 50.3/49.7% vs. 39.5/60.5%; $\chi^2 = 7.5$; df = 1; p = 0.006). The *ACE* DD genotype was more common among endurance-orientated athletes (32.8%) compared to those speed / power-orientated (19.1%).

ACTN3 genotype HWE calculations showed no deviation from expected frequencies in controls ($\chi^2 = 2.46$; df = 1; p = 0.117), but a deviation was observed in the total athlete group ($\chi^2 = 5.3$; df = 1; p = 0.02). The factors causing the deviation are not yet known. There were no significant allele or genotype frequency differences between the athlete group and the controls. Nevertheless, after grouping the athletes according to sex allele frequency, significant differences were determined between male athletes and male control groups (R/X: 58.2/41.8%, vs 66.5/33.5%; p = 0.03).

The distribution of the *PPARGC1A* ($\chi^2 = 1.03$; df = 1; p = 0.310) and *PPARA* ($\chi^2 = 0.24$; df = 1; p = 0.624) polymorphisms in athletes and in Lithuanian population was compatible with the HWE

(Table 2). The distribution of *PPARA* allele was significantly different between athletes and controls (G/C 77.5/22.5%, vs G/C 82.8/17.2%) $\chi^2 = 3.95$; df = 1; P = 0.046). There were no significant differences in allele or genotype frequency between the athlete group and the controls.

The average fitness characteristics of Lithuanian athletes in different sport categories are presented in Table 3. One-way ANOVA revealed significant differences in the mean values of the phenotypic variables among the different sports groups. The F statistics and p-value for each phenotypic variable are presented in Table 3. The multiple comparisons were carried out with Bonferroni adjustment. The mean values of the phenotypic variables in a specific sport group which appeared to be significantly different from the other groups in multiple testing ($p < 0.005$) are marked with stars. Athletes in different sports have very distinct fitness phenotypes. A small Roufier index is characteristic of endurance sports. Athletes in 'mix' sports have an elevated muscle mass and high indexes of the single muscular contraction power and anaerobic alactic muscular power.

One-way analysis of variance. ANOVA revealed significant differences in the mean values of the phenotypic variables in genotype groups of each individual gene in males, but none in females.

In male athletes, the average values of the BMI (F = 4.925, p = 0.008), FM (F = 5.087, p = 0.007), MM (F = 3.534, p = 0.032) and AAMP (F = 3.267, p = 0.041) differ significantly among *ACE* genotypes. The carriers of *ACE* DD allele have lower BMI, FM and MM. Athletes with *ACE* II genotype have a higher AAMP, which is important for excelling in the speed / power sports. The average values of SMCP (F = 3.360, p = 0.037) differ significantly among *ACTN3* genotypes. Athletes with *ACTN3* RX genotype have a significantly lower average SMCP. The MFMI (F = 5.038, p = 0.008) differs among *PPARGC1A* genotypes, and male athletes with *PPARGC1A* Ser482Ser genotype have a significantly higher MFMI, based on 9 observations in this group. The average MM (F = 4.999, p = 0.008) in male athletes – *PPARA* GG carriers is significantly lower. This implies that the *PPARA* GG genotype is less likely in the speed / power and mix sports requiring larger MM.

In female athletes, although the differences in fitness phenotypes were observed, none was identified as significant by ANOVA.

Logistic regression with backward variable selection. LR modelling results are summarized in Tables 4 and 5 for male and female athletes, respectively, including odds ratio, confidence intervals (CI) and p-values. The statistics of Hosmer–Lemeshow (HL) test of goodness of fit indicate how well the estimated LR model explains the observed data. Large p-values of HL statistics indicate a good correspondence between the observed data and the fitted LR model. The trends in genotype–phenotype relationship can be readily seen by examining the identified significant variables and the odds ratios associated with them.

Tables 4 and 5 summarize which phenotypic variables relate to each of the genotypes and a direction of the relationship. Whether the athletes with a specific genotype tend to have lower or higher values of a related phenotypic variable is indicated by the odds ratio being less or greater than 1. LR models were not identified for the genotypes represented by only few female athletes.

Body mass index. The BMI values ranged from low in the team and endurance sports to high in the speed / power and mix sports. The *PPARA* CC and GC genotypes were associated with high and the *PPARA* GG genotype with low BMI values. In males, increased BMI values were characteristic of the *ACTN3* RX genotype. Carriers of the *ACTN3* XX genotype in males and of the *ACTN3* RR genotype in females showed lower BMI values (Tables 4, 5). There was no evident trend in BMI changes to provide a link between the *ACTN3* genotype and the sports category in males or females.

Muscle mass. The MM values ranged from low in the team and endurance sports to high in the mix and speed / power sports. Male athletes with *ACE* II, *PPARA* CC and *PPARA* GC genotypes were associated with a higher MM (Table 4). Male athletes with the *ACE* DD and *PPARA* GG genotypes tended to be characterized by a lower muscle mass characteristic of endurance and team sports.

Fat mass. The FM values ranged from low in the endurance and team sports to high in the speed / power and mix sports. Male athletes with *ACE* ID and *ACTN3* RR genotypes

Table 3. **Actual average fitness phenotypes in different sport categories in Lithuanian elite athletes**

Physical fitness measures	Endurance sports (n = 64)	Speed / power group (n = 47)	Mix sports (n = 33)	Team sports (n = 49)	F	p
MM	39.8 ± 8.6	40.4 ± 9.2	47.7 ± 6.0*	32.9 ± 6.1*	24.14	0.000
FM	8.0 ± 2.3	8.7 ± 2.9	8.9 ± 5.1	8.1 ± 3.2	0.94	0.422
BMI	22.2 ± 2.1*	24.6 ± 4.1	25.0 ± 2.9	20.5 ± 2.2*	24.45	0.000
MFMI	5.3 ± 1.5	4.8 ± 1.0	6.3 ± 2.6*	4.5 ± 1.1	10.14	0.000
SMCP	2.3 ± 0.5	2.4 ± 0.3	2.6 ± 0.6*	2.2 ± 0.5	5.04	0.002
AAMP	1.56 ± 0.2	1.65 ± 0.2	1.69 ± 0.2*	1.55 ± 0.2	5.12	0.002
RI	3.2 ± 2.4*	4.5 ± 2.3	5.7 ± 3.7	5.4 ± 3.0	8.64	0.000

Values are means ± SD. FM, fat mass (kg); MM, muscle mass (kg), BMI, body mass index (kg/m^2); MFMI, muscle fat mass index; SMCP, single muscular contraction power (kgm/s/kg); AAMP, anaerobic alactic muscular power (kgm/s/kg); RI, Roufier index. The mean values found to be significantly different in a particular sport group by one-way ANOVA and multiple testing are marked by stars.

were associated with lower FM characteristics, while the carriers of *ACE* II and *ACTN3* XX genotypes were associated with a higher FM, linking these genotypes to the speed/power and mix sports (Table 4).

Muscle–fat mass index. The MFMI values ranged from low in the representatives of speed / power and team sports to high in the endurance and mix sports. The MFMI shows the muscle to fat mass ratio. In our study, in males the *ACE* DD, *PPARGC1A* Ser482Ser and *ACTN3* RX genotypes were associated with increased values of MFMI characteristics. The *ACE* II, *PPARGC1A* Gly482Ser and *ACTN3* RR genotypes were characterized by lower values of the MFMI, which were observed in speed / power and team sports (Table 4).

Single muscular contraction power. The SMCP values ranged from low in the endurance and team sports groups to high in the mix and speed / power sports groups. The SMCP reflects the performance of fast-contracting muscles. A high SMCP is required for improved performance in the speed / power and mix sports. Male athletes with *ACTN3, PPARGC1A* and *PPARA* genotypes were significantly associated with SMCP: the *ACTN3* RR, *PPARA* CC and *PPARGC1A* Gly482Gly genotypes were associated with increased values of SMCP (Table 4). Lower values of the SMCP were characteristic of the *ACTN3* RX genotype, suggesting that the X allele may have an effect towards a reduced speed / power capacity. In the female group, athletes with the *ACE* DD genotype were associated with a significantly lower SCMP (Table 5). Most of female athletes in our study practiced endurance sports, while only a few female athletes belonged to the speed / power or mix sports disciplines. The *ACE* D allele is likely to be advantageous for performance in endurance sports.

Anaerobic alactic muscular power. The AAMP values ranged from lower in the endurance and team sports to higher in the mix and speed / power sports. Male athletes with the *ACE* II genotype had characteristic increased AAMP values (Table 4). Decreased AAMP values were associated with the *ACE* ID and *PPARGC1A* Gly482Gly genotypes. In the female group, high AAMP values were linked to the *ACE* DD genotype and low values to the *ACE* II genotype (Table 5). Since the female athletes belonged to the endurance and team

Table 4. **Genotype–phenotype associations in male athletes in LR models**

Gene	Genotype	Logistic regression model			Hosmer–Lemeshow goodness of fit test (df = 8)	
		Variable	OR (CI)	p	Chi-square	p
ACE	DD	MM	0.934 (0.902, 0.968)	0.000	5.118	0.745
		MFMI	1.330 (1.046, 1.690)	0.020		
	ID	FM	1.255 (1.067, 1.476)	0.006	4.926	0.765
		AAMP	0.344 (0.160, 0.741)	0.006		
	II	FM	0.367 (0.211, 0.637)	0.000	2.096	0.987
		MM	1.220 (1.082, 1.375)	0.001		
		MFMI	0.301 (1.150, 0.606)	0.001		
		AAMP	20.332 (2.186, 189.131)	0.008		
ACTN3	RR	FM	0.854 (0.733, 0.995)	0.044	3.99	0.858
		MFMI	0.712 (0.557, 0.911)	0.007		
		SMCP	2.468 (1.149, 5.301)	0.021		
	RX	BMI	1.078 (1.003, 1.159)	0.041	4.857	0.773
		MFMI	1.351 (1.064, 1.716)	0.014		
		SMCP	0.276 (0.130, 0.587)	0.001		
	XX	BMI	0.854 (0.772, 0.945)	0.002	3.398	0.907
		FM	1.262 (0.953, 1.670)	0.104		
PPARGC1A	GlyGly	SMCP	2.472 (1.074, 5.688)	0.033	9.410	0.309
		AAMP	0.276 (0.080, 0.951)	0.041		
	GlySer	MM	1.022 (0.993, 1.052)	0.133	5.763	0.674
		MFMI	0.799 (0.644, 0.990)	0.040		
	SerSer	BMI	0.801 (0.721, 0.891)	0.000	7.314	0.503
		MFMI	1.786 (1.183, 2.696)	0.006		
		RI	0.684 (0.498, 0.940)	0.019		
PPARA	GG	BMI	1.349 (1.143, 1.593)	0.000	8.371	0.398
		MM	0.859 (0.781, 0.945)	0.002		
	GC	BMI	0.751 (0.636, 0.888)	0.001	8.088	0.428
		MM	1.152 (1.046, 1.268)	0.004		
	CC	BMI	0.504 (0.318, 0.798)	0.003	8.416	0.394
		MM	1.258 (1.025, 1.543)	0.028		
		SMCP	4.906 (1.022, 23.543)	0.047		
		RI	0.757 (0.571, 1.003)	0.053		

Table 5. **Genotype–phenotype associations in female athletes in LR models**

Gene	Genotype	Logistic regression model			Hosmer–Lemeshow goodness of fit test (df = 8)	
		Variable	OR (CI)	p	Chi-square	p
ACE	DD	SMCP	0.034 (0.002, 0.525)	0.015	7.896	0.444
		AAMP	267.7 (3.571, 20068)	0.011		
		RI	0.713 (0.531, 0.958)	0.125		
	ID	BMI	0.985 (0.958, 1.013)	0.286	3.642	0.888
	II	AAMP	0.483 (0.295, 0.788)	0.004	9.255	0.321
ACTN3	RR	BMI	0.968 (0.940, 0.997)	0.030	14.747	0.064
	RX	SMCP	1.182 (0.890, 1.570)	0.248	4.262	0.833
	XX	Not identified				
PPARGC1A	GlyGly	BMI	0.952 (0.890, 1.018)	0.151	10.861	0.210
	GlySer	MFMI	0.936 (0.811, 1.080)	0.365	8.675	0.370
	SerSer	Not identified				
PPARA	GG	FM	1.051 (0.993, 1.112)	0.084	9.376	0.311
	GC	BMI	0.960 (0.931, 0.990)	0.009	3.628	0.889
	CC	Not identified				

sports, higher AAMP values in the endurance sports link the *ACE* D allele to the endurance and *ACE* I allele to the team sports.

Roufier index. A low RI indicates good physical fitness and training. Lower values of this phenotypic variable (<5) are required in endurance sports. Male athletes with the *PPARGC1A* Ser482Ser genotype and female athletes with the *ACE* DD genotype showed a decreased RI (Table 4, 5).

DISCUSSION

The standard values of fitness phenotypes in different sports categories, coupled with the results of logistic regression analysis suggested the genotypes / alleles that may influence predisposition towards a specific sports category. Combined results of ANOVA and LR modeling show that all investigated polymorphisms have associations with one or several fitness phenotypes in Lithuanian athletes: (i) according to the *ACE* genotype, male athletes with the II genotype had higher MM and AAMP compared to the DD-genotyped athletes; power-orientated *ACE* II genotype athletes had a significantly higher AAMP than *ACE* II genotype athletes from endurance and mix groups; (ii) male athletes with the *PPARA* CC, *PPARGC1A* Gly482Gly and *ACTN3* RR (female with the *ACTN3* RX) genotypes were associated with increased values of SMCP; (iii) male athletes with the *PPARA* CC and *PPARGC1A* Gly482Ser genotypes had a significantly higher MM; (iiii) male athletes with the *ACE* DD, *ACTN3* RX, *PPARGC1A* Ser482Ser genotypes were associated with higher MFMI values, while there were no significant differences among the female athlete groups.

Genetic diversity appears to make a significant contribution to performance-related phenotypes (4).

Elite endurance athletic performance depends on the complex interactions between the cardiovascular and pulmonary systems, muscular metabolism and musculoskeletal adaptations. The relationship between the *ACE* I/D alleles and the speed / power, or endurance, remains undetermined in the literature (4, 8, 9, 11, 13). We conclude that in Lithuanian elite endurance athletes the frequency of the *ACE* D allele and DD genotype seems to be higher than in speed / power athletes, suggesting a positive association between the D allele and the likelihood of becoming an elite endurance athlete. The vertical jump test and stair-climb test, quantified by the higher SMCP and AAMP values, in the *ACE* II genotype athletes relate the I allele to speed and power. In males with the *ACE* II genotype it can be associated with a higher speed / power capacity because the required high AAMP values in the speed / power sports are characteristic of this genotype. In females with the *ACE* DD genotype is assumed to be related to a higher endurance performance because a low Roufier index was characteristic of this genotype. These findings were in agreement with data from several reports stating that the *ACE* DD genotype is associated with endurance and the *ACE* II with speed / power sports (2, 9, 10, 12).

Variation in genes involved in lipid, glucose and energy homeostasis is expected to have a role in physical performance. The activity of transcription factor PGC-1a is influenced by the *PPARGC1A* Gly482Ser polymorphism (3, 13, 18). The *PPARGC1A* 482Ser allele is associated with a lower metabolism (3, 18). We have found that the *PPARGC1A* genotype Gly482Gly is more frequent in endurance and mixed athletes. In our study, *PPARGC1A* Gly482Gly genotype athletes showed a strong association with a higher SMCP and a low AAMP. However, in males the *PPARGC1A* Ser482Ser genotype was associated with a low Roufier index indicating a higher endurance. It is possible that a genetic predisposition towards poorer performance in sports is compensated by training or other factors such as a chance result caused by a small number of male athletes with the *PPARGC1A*

Ser482Ser genotype. The *PPARGC1A* Gly482Ser variant could be in linkage disequilibrium with an unknown variant elsewhere in the gene, which could explain the discrepancies among the results of association studies. It is possible that the effect of the coactivator *PPARGC1A* during physical strain is expressed through other transcription factors such as PPARA or other.

The *PPARA* gene is important in glucose and lipid metabolism (3, 17, 18, 22). It is more expressed in type I (slow-twitch) muscle fibres as compared to type II (fast-twitch) fibres (8, 22, 24). The *PPARA* G allele is assumed to be the endurance allele and the C allele is the speed / power allele (1, 8, 24). Our results support these assumptions. Results of the present study imply that the *PPARA* C allele is more common in the athlete group than in the general population of Lithuania. The *PPARA* GG genotype was more frequent among athletes in endurance and team sports than in speed / power and mix sports. Males in the speed / power sport category have high SMCP and AACP values. The *PPARA* CC genotype was significantly associated with high SMCP values, suggesting that the C allele determines speed and power. Thus, success in sports can be attributed to the *PPARA* of alleles associated with a certain physical phenotype.

The ACTN3 protein is assumed to be responsible for the ability of fast muscle fibres to generate power rapidly (3, 13–15). The *ACTN3* XX genotype is negatively associated with elite sprint athlete status (2, 8, 13, 14). Athletes with the *ACTN3* RR and RX genotypes have the ACTN3 protein in their fast contracting muscle cells; therefore, it is likely that these individuals will show high performance in sports based on speed / power (2, 8, 13, 14). Results of the present study imply that the *ACTN3* RR genotype is significantly associated with high SMCP values, suggesting that these genotypes may be linked to higher achievements in speed / power sports.

The effects of the *ACE*, *ACTN3, PPARA* and *PPARGC1A* gene variants were different on male and female athletes.

Only the fittest and best adapted athletes remain in sports. We explored a small number of elite athletes that were available in each sports discipline. Nevertheless, elite athletes know the prime determinants of success in a well-defined sports category. Their distinctive fitness phenotypes are associated with the specific genotypes in a significant way.

Although more studies are needed, these preliminary data suggest a possibility to use some of these genetic variants in an individually prepared prescription of lifestyle or exercise for health and sports performance.

CONCLUSIONS

The above findings suggest associations between *ACE*, *ACTN3*, *PPARGC1A*, *PPARA* polymorphisms and physical performance in Lithuanian athletes. In particular, the *ACE* II, *PPARA* CC, *ACTN3* RR genotypes appeared to be related to speed / power sports and the *ACE* DD, *PPARA* GG to endurance sports. *PPARGC1A* Ser482Ser may not be critical for but rather additive to endurance performance. Our findings confirm the polygenic nature and a classic complex trait of physical performance. The identified genotype–phenotype associations facilitate the application of improved training strategies to athletes developing the fitness phenotypes optimal for a specific sport discipline.

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Valentina Ginevičienė, Erinija Pranckevičienė, Kazys Milašius, Vaidutis Kučinskas

DIDELIO MEISTRIŠKUMO LIETUVOS SPORTININKŲ FIZINIO PAJĖGUMO FENOTIPO SĄSAJA SU GENOTIPU

S a n t r a u k a

Įvadas. Tyrimo tikslas – ištirti genotipo poveikį fizinio pajėgumo fenotipui didelio meistriškumo Lietuvos sportininkų grupėse.

Medžiaga ir metodai. Ištirti 193 (152 vyrai ir 41 moteris) didelio meistriškumo Lietuvos sportininkai bei 250 kontrolės asmenys pagal polimorfizmus, dažniausiai siejamus su fiziniu pajėgumu: *ACE* (rs1799752), *ACTN3* (rs1815739), *PPARGC1A* (rs8192678), *PPARA* (rs4253778). Sportininkai buvo suskirstyti į keturias grupes: ištvermės, greičio / jėgos, aerobinio / anaerobinio pajėgumo ir žaidėjų. Genotipo-fenotipo asociacija nustatyta taikant dispersinę analizę ir logistinės regresijos modelį.

Rezultatai. Nustatyta, kad *ACE* I alelis buvo dažnesnis sportininkų grupėje negu kontrolės grupėje, nors tik vyriškos lyties sportininkų alelių dažniai skyrėsi nuo kontrolinės grupės vyrų. *PPARA* C alelis dažnesnis sportininkų grupėje nei bendroje Lietuvos populiacijoje. Tiriant *ACTN3* ir *PPARGC1A* vieno nukleotido polimorfizmus, alelių ar genotipų dažnių pasiskirstymas tarp sportininkų grupių ir kontrolės reikšmingai nesiskyrė. Mūsų tyrimais nustatyta, kad *ACTN3* RR genotipas yra susijęs su vienkartiniu raumenų susitraukimo galingumu; *PPARGC1A* Ser482Ser – su raumenų riebalų masės indeksu; *PPARA* CC ir *ACE* II genotipai yra susiję ir su raumenų mase, ir su vienkartiniu raumenų susitraukimo galingumu. Tirti genetiniai variantai turi skirtingą įtaką vyrų ir moterų fenotipiniams požymiams.

Išvados. Nustatyta, kad Lietuvos sportininkams, turintiems *ACE* II, *ACTN3* RR, *PPARA* CC genotipus, būdingas greitis ir jėga, o *ACE* DD ir *PPARA* GG – ištvermė. *PPARGC1A* Ser482Ser genotipas galbūt nėra lemiamas veiksnys, tačiau bendras jo poveikis turi įtakos į ištvermę orientuotai fizinei veiklai.

Raktažodžiai: fizinis pajėgumas, genetiniai variantai, sporto kategorijos