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# Genetic Variation of the Human *ACE* and *ACTN3* Genes and Their Association With Functional Muscle Properties in Lithuanian Elite Athletes

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***Key words:*** *ACE; ACTN3; physiological parameters; physical performance.*

***Summary.*** *Background and Objective. Based on the results of many studies, the angiotensin-converting enzyme (ACE) and the α-actinin-3 (ACTN3) genes are considered strong candidate genes associated with human physical performance. On the other hand, the data regarding the association of the ACE I/D and ACTN3 R/X polymorphisms with human physical performance in different populations have been conflicting. The objective of our research was to evaluate the significance of these genetic variants on muscle performance phenotype in Lithuanian athletes.*

*Material and Methods. The study involved 193 Lithuanian elite athletes and 250 controls from the general Lithuanian population. Genotyping was performed by polymerase chain reaction and/or restriction fragment length polymorphism analysis. Anthropometric measurements and muscle strength (grip strength and vertical jump) were measured.*

*Results. It was determined that ACE I/I and I/D genotypes were more frequent in the athlete group compared with the general Lithuanian population. The results of grip strength and vertical jump were better in the athletes with the ACE I/I and ACTN3 X/X genotype compared with the athletes with ACE D/D and ACTN3 R/R, respectively.*

*Conclusions. The ACE I and ACTN3 X alleles determine speed and power for Lithuanian athletes. In line with other researchers, it can be confirmed that the absence of a functional ACTN3 in fast-twitch muscle fibers is compensated. Lithuanian athletes who are carriers of the ACE I/I and I/D as well as ACTN3 X/X and R/X genotypes have the potential to achieve better results in power-requiring sports; therefore, the analyzed polymorphisms of these genes might be used as the criteria for the sport type selection.*

## Introduction

Although the making of an elite athlete is complex and includes a range of environmental and behavioral factors, genetic predisposition to athleticism is also important (1, 2). The information regarding the association of handgrip strength, short-term explosive power, and various anthropometric variables with the *ACE* and *ACTN3* genotypes in elite athletes is lacking; thus, the present study was conducted (1). The angiotensin-converting enzyme (*ACE*) and α-actinin-3 (*ACTN3*) genes are two of the most studied "performance genes" and both have been associated with sprint as well as other power phenotypes and elite performance (2). The data regarding the association of polymorphisms of the *ACE* I/D (rs1799752) and *ACTN3* R577X (rs1815739) genes with human physical performance have been conflicting (1, 3). Circulating ACE exerts a tonic regulatory function in circulatory homeostasis (1). A polymorphism of the human *ACE* gene (17q22-q24) has been identified in which the presence (insertion, I allele) rather than the absence (deletion, D allele) of a 287-bp Alu-sequence insertion fragment is associated with lower serum and tissue ACE activity (4). An excess of the I allele has been associated with some aspects of endurance performance (3, 5). Similarly, several studies have shown the *ACE* D allele to be associated with greater strength and muscle volumes at baseline and an increased percentage of fast-twitch muscle fibers. In addition, the D allele was associated with the elite power athlete status (5).

The human *ACTN3* gene encodes the protein α-actinin-3, a component of the contractile apparatus in fast-twitch skeletal muscle fibers (1, 6, 7). A common genetic variation in the *ACTN3* gene results in the replacement of arginine (R) with a stop codon (X) at amino acid 577 (p.R577X; C→T transition at position 1747 in exon 16, 11q13-q14) (1, 6). Ho-

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mozygosity for the nonsense mutation, X/X, within *ACTN3* results in the deficiency of α-actinin-3; however, it does not result in an abnormal muscular phenotype (1, 8). It has also been suggested that the X allele may confer an advantage during endurance events (1, 3, 7). Several case-control studies have determined that the *ACTN3* R/R genotype is overrepresented, and the *ACTN3* X/X genotype is under-represented in power-oriented (including sprint) athletes in comparison with controls. The hypothesis that *ACTN3* R allele may confer some advantage in power performance events was supported by several cross-sectional studies in nonathletes including mouse models of the *ACTN3* deficiency (5).

Among human performance tests, strength and anaerobic power are much more indicative of muscle properties. Only muscular power, which is demonstrated by the stair climb and vertical jump tests (jumping is a very explosive movement), also showed a significant genetic component. Static power tends to have higher heritabilities than muscular endurance (9). Although power phenotypes are under moderately strong to strong genetic control, there is still a long way to go before conclusive results concerning genetic polymorphisms associated with these phenotypes can be presented (7, 9). This study investigated the association between the *ACE* I/D and *ACTN3* R/X polymorphisms and muscle power properties in Lithuanian athletes.

## Material and Methods

The study followed recent recommendations for the studies on replicating genotype-phenotype associations (10). The Lithuanian Bioethics Committee approved the study, and written informed consent was obtained from each participant.

*Subjects.* The study involved 193 athletes (mean age, 22.0; SD, 6.3 years) and 250 healthy unrelated controls (167 men and 83 women; mean age, 36.2; SD, 7.2 years) from 6 ethnolinguistic Lithuanian groups. All athletes and controls were Caucasians. A group of 193 elite athletes (152 men and 41 women) designated as Olympic candidates and athletes who participated in international competitions with experience of not less than 5 years in their sports categories were studied. The athletes were prospectively stratified into three groups according to the event duration and distance spanning a spectrum from the endurance-oriented to the power-oriented athletes. The endurance-oriented group (n=77) included very long (race duration, >30 min), long (race duration, 5–30 min), and middle (race duration, 45 s to 5 min) distance athletes; the power-oriented group (n=51) included sprint and other power athletes with predominantly anaerobic energy production. The mixed group (n=65) comprised athletes whose sports utilized mixed anaerobic and aerobic energy production.

*Anthropometric Measurements.* Body height was measured to the nearest 0.01 m with the subjects standing with their back to a wall-mounted stadiometer. Weight was measured to the nearest 0.1 kg with calibrated scales. Body mass index (BMI, in $kg/m^2$) was calculated as weight (in kg) divided by height (in $m^2$). Highly trained athletes may have a high BMI because of increased muscularity rather than increased body fat. Total body fat mass (FM) was determined by measuring the size of the thickest places of the forearm, humeral area, thigh, and calf using a caliper to measure the thickness of the skin, thus, determining the amount of subcutaneous fat.

*Muscle Strength Measurements.* Muscle power has been considered as an important physical ability particularly responsible for success of rapid movements. Power can be measured using different testing methods and in different upper or lower extremity muscle groups, but still strong relationships are observed among various strength measurements. Such relationships suggest that any strength measurement may reflect the pattern of power within an individual. A maximum vertical jump test is a good estimate of lower extremity power output. Short-term explosive muscle power (STEMP) was measured by asking the subject to perform a maximal vertical jump (on the contact platform). The power output expressed per unit body weight was measured according to the method proposed by Bosco and modified by Linthorne (11). Maximal isometric power of the forearm muscles (handgrip test according to the procedures described by Mathiowetz et al.) was measured with an adjustable mechanical hand dynamometer and expressed in kg (12).

*Genotyping.* Genomic DNA was extracted from peripheral blood leukocytes by the standard phenol-chloroform extraction method. Polymerase chain reaction (PCR) was used to detect the I and D alleles of the *ACE* (rs1799752) gene according to the method described by Rigat et al. (1990) using the upstream primer 5′-CTGGAGACCACTCCCATCCTTTCT-3′ and the downstream primer 5′-GATGTGGCCATCACATTCGTCAGAT-3′. This method yields PCR fragments of 190 bp and 477 bp in the presence of the D and the I alleles, respectively. Reaction products were visualized by electrophoresis on a 2% agarose gel and identified by ethidium bromide staining (Fig. 1).

Genotyping of the *ACTN3* (rs1815739) polymorphism was performed using PCR. The resulting PCR products were genotyped by restriction fragment length polymorphism (RFLP). The *ACTN3* R/X polymorphism was amplified using the PCR forward primer 5′-CTGTTGCCTGTGGTAAGTGGG-3′ and the reverse primer 5′-TGGTCACAGTATGCAGGAGGG-3′. The amplified fragment subsequently underwent digestion by *DdeI* restriction enzyme described by Mills et al. (13). Digested PCR fragments

(108 bp, 97 bp, and 86 bp fragments for R allele and 205 bp and 86 bp for X allele) were separated by 8% polyacrylamide gel electrophoresis (Fig. 2).

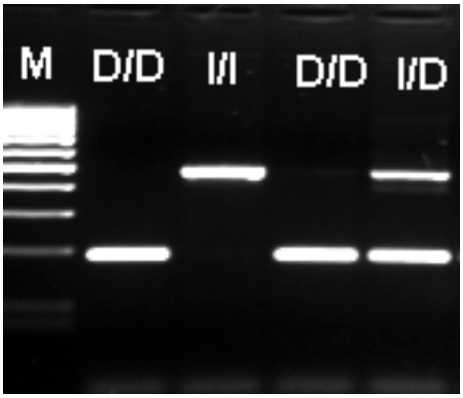


*Fig. 1.* Genotypes for the *ACE* I/D polymorphism

D/D, 190 bp; I/D, 190 bp and 477 bp; I/I, 477 bp; M, DNA molecular size standard (*GeneRuler* 100 bp).

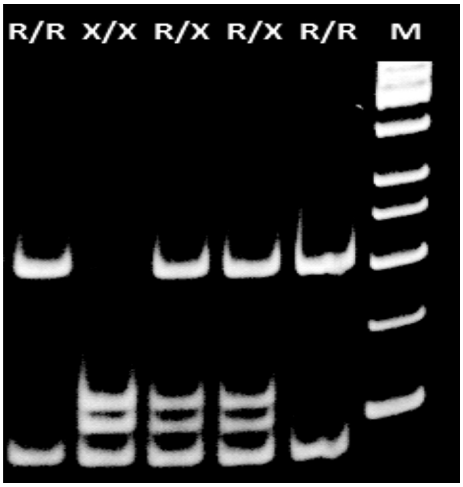


*Fig. 2.* Genotypes for the *ACTN3* R/X polymorphism

R/R, 205 bp and 86 bp; R/X, 205 bp, 108 bp, 97 bp, and 86 bp; X/X, 108 bp, 97 bp, and 86 bp; M, DNA molecular size standard (*GeneRuler* 50 bp).

*Statistical Analysis*. Genotype frequencies of the athletes were tested for compatibility with the Hardy-Weinberg equilibrium (HWE). The chi-square test was used to assess the fit of the observed genotype frequencies to the HWE. Differences in genotype and allele frequencies between groups were assessed by the chi-square test with expected values equal for all categories. One-way ANOVA was used for the comparison of the mean of the phenotypic variables. The level of significance was set at 0.05. The statistical software package SPSS (v.13) was used to obtain the results.

## Results

The distribution of the *ACE* and *ACTN3* polymorphisms in 193 Lithuanian athletes and 250 healthy untrained individuals was compared. The data obtained for both genotype and allele distributions are presented in Table 1.

The *ACE* genotype HWE calculations showed no deviation from expected frequencies in the athlete group (II/ID/DD, 25.9%/47.7%/26.4%; $\chi^2$=0.42, *P*=0.52), but a deviation was observed in controls (II/ID/DD, 23.6%/38.0%/38.4%; $\chi^2$=12.43, *P*=0.0004). In the control group, significant differences in allele frequencies were determined between men and women (I/D, 39.5%/60.5% vs. 48.8%/51.2%; $\chi^2$=3.901, *P*=0.048). *ACE* I/D polymorphism allele frequencies were significantly different between the athlete and control groups (I/D, 49.7%/50.3% vs. 42.6%/57.4%; *P*=0.034) (Table 1). Similar significant differences were obtained comparing allele frequencies between male athletes and controls (I/D, 50.3%/49.7% vs. 39.5%/60.5%; *P*=0.006) as well as comparing the *ACE* genotypes in power-oriented athletes and controls (II/ID/DD, 23.5%/56.9%/19.6% vs. 23.6%/38.0%/38.4%; *P*=0.019) (Table 1). The *ACE* D/D genotype was more common among endurance-oriented athletes (31.2%) compared with power-oriented athletes (19.6%).

*Table 1.* Genotype Frequencies of *ACE* I/D and *ACTN3* R/X Polymorphisms in Athletes and Controls

| Group | N | *ACE* Genotype, N (%) | | | *ACE* Allele | | *ACTN3* Genotype, N (%) | | | *ACTN3* Allele | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | I/I | I/D | D/D | I | D | R/R | R/X | X/X | R | X |
| Endurance group | 77 | 21 (27.2) | 32 (41.6) | 24 (31.2) | 0.481 | 0.519 | 20 (26.0) | 49 (63.6) | 8 (10.4) | 0.578 | 0.422 |
| Power group | 51 | 12 (23.5)‡ | 29 (56.9)‡ | 10 (19.6)‡ | 0.520 | 0.480 | 19 (37.3) | 24 (47.1) | 8 (15.7) | 0.608 | 0.392 |
| Mixed group | 65 | 17 (26.2) | 31 (47.7) | 17 (26.2) | 0.5 | 0.5 | 20 (30.8) | 36 (55.4) | 9 (13.8) | 0.585 | 0.415 |
| Total athletes | 193 | 50 (25.9)* | 92 (47.7)* | 51 (26.4)* | 0.497† | 0.503† | 59 (30.6) | 109 (56.5) | 25 (12.9) | 0.588 | 0.412 |
| Control subjects | 250 | 59 (23.6)*‡ | 95 (38)*‡ | 96 (38.4)*‡ | 0.426† | 0.574† | 98 (39.2) | 126 (50.4) | 26 (10.4) | 0.644 | 0.356 |

*$\chi^2$=7.35, *df*=2, *P*=0.025 for *ACE* genotype frequencies comparing total athletes and control subjects; †$\chi^2$=4.48, *df*=1, *P*=0.034 for *ACE* allele frequencies comparing total athletes and control subjects; ‡$\chi^2$=7.91, *df*=2, *P*=0.019 for *ACE* genotype frequencies comparing power athletes and control subjects.

The *ACTN3* genotype HWE calculations showed no deviation from expected frequencies in controls (RR/RX/X/X, 39.2%/50.4%/10.4%; $\chi^2$=2.46, *P*=0.12), but a deviation was observed in the athlete group (RR/RX/X/X, 30.6%/56.5%/12.9%; $\chi^2$=5.3, *P*=0.02), as Table 1 demonstrates. There were no significant differences in allele or genotype frequencies between the athlete group as a whole and the control group. Nevertheless, after dividing athletes according to sex, significant differences were determined between male athlete and control groups (R/X, 58.2%/41.8% vs. 66.5%/33.5%; *P*=0.03). There were no significant differences between the sports groups.

The phenotypic variables of the control group were not measured due to various limitations. Male and female athletes were analyzed separately given the known gender-specific influences of the *ACE* and *ACTN3* genotypes on phenotypic measures. It was found that the mean values of all analyzed variables were significantly different with respect to gender (*P*<0.01) and sports groups (*P*<0.04). However, the mean differences between genders within sports groups showed less pronounced results. In the group of endurance-oriented athletes, only the average values of all phenotypic variables were significantly different (*P*<0.04). The mean value of anaerobic power variables, such as STEMP or left and right handgrip strength, was significantly higher for

*Table 2.* Descriptive Summary of Phenotypic Characteristics of Lithuanian Athletes With Respect to Gender and Athlete Genotypes

| Genotype | Gender | Height, cm | Weight, kg | BMI, kg/m$^2$ | RGS, kg | LGS, kg | Fat mass, kg | Muscle mass, kg | STEMP, W |
|---|---|---|---|---|---|---|---|---|---|
| | | | | *ACE* | | | | | |
| I/D | Male (n=17) | 179.6 (9.2)* | 76.9 (15.2)* | 23.8 (3.6) | 50.0 (9.5)* | 48.5 (9.3)* | 8.3 (2.7)* | 41.9 (8.9)* | 1836.8 (486.0)* |
| | Female (n=75) | 174.9 (6.6)* | 68.4 (13.9)* | 22.1 (3.3) | 35.6 (8.4)* | 34.6 (10.4)* | 11.6 (5.8)* | 36.3 (7.4)* | 1554.2 (377.6)* |
| I/I | Male (n=11) | 180.4 (7.6) | 75.6 (12.9) | 23.0 (2.9) | 50.7 (8.9)* | 49.2 (9.8)* | 7.6 (1.8)* | 41.6 (7.9) | 1888.3 (548.6) |
| | Female (n=39) | 174.4 (7.9) | 67.4 (12.3) | 22.2 (2.9) | 34.3 (6.9)* | 35.0 (7.3)* | 10.9 (5.2)* | 35.1 (5.8) | 1683.2 (306.8) |
| D/D | Male (n=13) | 179.1 (11.5) | 70.6 (13.9) | 21.7 (3.2) | 45.6 (8.7)* | 44.5 (9.3)* | 6.8 (1.8)* | 37.5 (8.1) | 1628.3 (425.9) |
| | Female (n=38) | 172.5 (9.1) | 64.4 (14.4) | 21.9 (3.0) | 37.8 (7.3)* | 37.2 (7.2)* | 9.3 (3.0)* | 33.4 (11.8) | 1503.9 (421.8) |
| | | | | *ACTN3* | | | | | |
| R/X | Male (n=24) | 179.7 (9.4)* | 75.5 (15.2)* | 23.2 (3.6) | 49.5 (9.6)* | 48.0 (9.3)* | 7.7 (1.7)* | 41.3 (9.0)* | 1752.2 (509.4) |
| | Female (n=85) | 173.8 (7.9)* | 66.7 (10.8)* | 22.0 (2.4) | 34.9 (6.8)* | 33.0 (7.9)* | 10.4 (3.8)* | 34.1 (8.5)* | 1575.6 (370.7) |
| R/R | Male (n=13) | 179.6 (9.1) | 73.9 (12.7) | 22.6 (2.8) | 48.9 (8.8)* | 47.3 (9.9)* | 7.6 (1.7) | 40.0 (8.0) | 1840.2 (520.0) |
| | Female (n=46) | 174.2 (7.9) | 69.9 (17.9) | 22.6 (4.2) | 38.5 (8.5)* | 41.1 (7.3)* | 11.2 (6.8) | 36.5 (9.6) | 1606.7 (405.7) |
| X/X | Male (n=4) | 179.8 (10.5) | 75.2 (13.9) | 23.3 (4.0) | 47.9 (9.6)* | 47.0 (10.5)* | 8.2 (2.4) | 40.2 (10.0) | 1890.1 (555.3) |
| | Female (n=21) | 174.8 (7.2) | 67.9 (12.4) | 20.9 (2.1) | 33.8 (9.6)* | 32.3 (9.5)* | 10.7 (4.4) | 36.0 (6.9) | 1446.3 (338.1) |

Data are mean (SD). *$P$<0.05 between gender groups. BMI, body mass index; RGS, right grip strength; LGS, left grip strength, STEMP, short-term explosive muscle power.

*Table 3.* Descriptive Summary of Phenotypic Characteristics of Lithuanian Athletes With Different *ACE* I/D and *ACTN3* R/X Polymorphisms Genotypes in the Sports Groups

| Genotype | Group | RGS, kg | LGS, kg | Muscle mass, kg | STEMP, W |
|---|---|---|---|---|---|
| *ACE* I/D | Endurance (n=32) | 48.0 (10.8) | 46.5 (9.8) | 42.5 (8.8) | 1801.4 (498.9) |
| | Power (n=29) | 53.5 (8.6) | 52.7 (8.5) | 45.6 (8.2) | 2013.4 (453.2) |
| | Mixed (n=31) | 40.9 (8.7)*† | 38.9 (9.9)*† | 34.8 (5.8)*† | 1553.2 (376.9)† |
| *ACE* I/I | Endurance (n=21) | 50.4 (10.3) | 50.1 (8.4) | 41.8 (5.9) | 1902.8 (465.8) |
| | Power (n=12) | 52.0 (10.5) | 50.5 (11.0) | 45.6 (9.8) | 2103.4 (594.5) |
| | Mixed (n=17) | 39.5 (7.7)*† | 37.9 (9.5)*† | 34.5 (4.8)*† | 1585.8 (395.0)† |
| *ACE* D/D | Endurance (n=24) | 45.5 (8.1) | 44.8 (8.7) | 39.0 (10.0) | 1592.4 (449.8) |
| | Power (n=10) | 47.5 (7.7) | 46.2 (7.5) | 40.8 (9.9) | 1815.2 (500.3) |
| | Mixed (n=17) | 38.7 (9.1)*† | 37.5 (9.4)*† | 30.3 (6.7)*† | 1473.9 (544.0) |
| *ACTN3* R/X | Endurance (n=49) | 48.1 (9.9) | 46.7 (9.3) | 41.8 (9.3) | 1727.8 (492.7) |
| | Power (n=24) | 52.8 (9.4) | 51.3 (9.7) | 44.7 (9.1) | 1987.7 (441.1) |
| | Mixed (n=36) | 39.4 (9.3)*† | 37.5 (9.8)*† | 33.6 (6.0)*† | 1510.7 (418.4)† |
| *ACTN3* R/R | Endurance (n=20) | 46.9 (10.1) | 47.1 (8.6) | 38.7 (6.8) | 1833.7 (488.3) |
| | Power (n=19) | 50.6 (9.7) | 50.1 (9.6) | 44.6 (9.5) | 1913.3 (529.9) |
| | Mixed (n=20) | 42.5 (6.8)† | 40.9 (8.9)† | 34.7 (5.9)† | 1625.5 (472.7) |
| *ACTN3* X/X | Endurance (n=8) | 48.6 (10.7) | 48.3 (11.2) | 43.9 (7.5) | 1810.8 (463.5) |
| | Power (n=8) | 52.5 (6.8) | 51.5 (7.4) | 44.6 (8.3) | 2215.4 (575.6) |
| | Mixed (n=9) | 36.8 (7.8)*† | 35.4 (9.4)*† | 31.1 (6.3)*† | 1474.3 (357.5)† |

*P* values adjusted for multiple comparisons using the Bonferroni test. *$P$<0.05, mixed group versus endurance; †$P$<0.05, mixed group versus endurance group. RGS, right grip strength; LGS, left grip strength; STEMP, short-term explosive muscle power.

men in all groups (with the exception of STEMP in the power-oriented group).

Considering the mean differences of phenotypic variables for the *ACE* and *ACTN3* genotypes with respect to gender and sports group, one-way ANOVA was applied as well. In order to perform it, the dataset was split according to the *ACE* and *ACTN3* genotypes. This means that each genotype was analyzed separately, which allowed comparing the average values of the phenotypic variables using one-way ANOVA when initially the gender and later on the sports group were selected as independent variables. The results for the genders and sports groups are demonstrated in Table 2 and Table 3, respectively.

Considering ANOVA results for genders, the left and right handgrip was significantly higher for male athletes with all the *ACE* and *ACTN3* genotypes ($P<0.001$). The other significant anaerobic power variable STEMP was significantly higher for male athletes of the *ACE* I/D heterozygous genotype only ($P<0.001$). Male and female athletes with the *ACE* I/I genotype had higher handgrip strength and STEMP compared with male and female athletes having the *ACE* D/D genotype.

According to the *ACE* gene polymorphism from the perspective of sports groups, the right and left handgrip strength was significantly greater for endurance-oriented and power-oriented groups compared with the mixed group for all genotypes ($P<0.05$). The left handgrip strength was significantly greater in the power-oriented group compared with the endurance-oriented group for the *ACE* I/D genotype ($P<0.04$). STEMP appeared to be significantly different only between power-oriented and mixed groups for the *ACE* I/I and I/D genotypes ($P<0.02$). Athletes with the *ACE* I/I and I/D genotype in the power-oriented group had higher muscle mass, handgrip strength, and STEMP compared with athletes with the D/D genotype.

As to the *ACTN3* gene polymorphism, significant differences were found between the power-oriented and mixed groups for the short-term explosive muscle power for the *ACTN3* R/X and X/X genotypes ($P<0.01$) as well as for the right and left handgrip strength for R/R homozygous genotype ($P<0.01$). In addition, the mean values of right and left handgrip strength were significantly higher for the athletes with the *ACTN3* R/X and X/X genotype in the endurance-oriented group compared with the mixed group ($P<0.001$). All of the athletes in the speed or power-oriented group irrespective of the genotype had higher muscle mass, handgrip strength, and STEMP compared with those from other sports groups. Athletes with the *ACTN3* X/X genotype in the speed or power-oriented group had higher STEMP than the athletes with the R/R and R/X genotype in all sports groups ($P<0.05$). STEMP values of the athletes with the *ACTN3* X/X genotype were higher compared with those of the athletes with the *ACTN3* R/R and R/X genotype.

## Discussion

It has already been shown that many variants that have a significant association with physical performance in several studies of one population may not necessarily have the same association in another. Phenotypes that are related to power performance were analyzed to begin creating a chain of evidence linking the studied polymorphisms with success in power-oriented sports. This study investigated the association between the *ACE* I/D and *ACTN3* R/X polymorphisms and muscle strength properties in Lithuanian athletes.

The studied Lithuanian population (according to the *ACE* I/D polymorphism) from which the control group was constructed was not in accordance with the Hardy-Weinberg equilibrium despite the fact that the control group was selected from healthy unrelated Caucasian subjects from geographically distinct regions of Lithuania. To date, controversial results have been reported regarding the association of the *ACE* I/D polymorphism with athletic ability. Several investigations have found no association of the *ACE* genotype with isometric and dynamic strength (5, 14). To our knowledge, no studies have examined the influence of the *ACE* genotype on high velocity strength and the contractile properties of human skeletal muscle, which is of great interest given the potential influence of the *ACE* genotype on the fiber type composition. According to our results, the *ACE* I/D polymorphism I allele was more frequent in the Lithuanian power-oriented group compared with other sports groups and the control group indicating association with power. The latter was also reflected in the vertical jump test where athletes with the *ACE* I/I and I/D genotype showed higher STEMP confirming the conclusion that the I allele determined speed and power.

The other candidate gene considered in this study was the *ACTN3* and its R/X polymorphism. The known biology of the R577X polymorphism would suggest a recessive model with the $\alpha$-actinin-3 deficiency present in X/X homozygous individuals only. The data obtained from a recently regenerated *ACTN3* knockout mouse model showed altered muscle fiber morphology in X/X carriers, thus, giving an opportunity to analyze the underlying mechanisms, which cause the functional differences observed in the present and previous studies (15). The *ACTN3* X/X genotype was more frequent in the Lithuanian athlete group compared with the control group, which contradicts the findings of other researchers; furthermore, the studied athlete group was not in concordance with the Hardy-Weinberg equilibrium. Our results indicate that *ACTN3* X/X homozygous athletes have typically higher short-term explosive power than the athletes with the R/R genotype.

Therefore, it can be concluded that the absence of a functional ACTN3 in fast-twitch muscle fibers might be compensated most likely by ACTN2 or other enzymes that take part in anaerobic muscle metabolism. Although some laboratories suggest genetic testing for choosing an appropriate sports career, the ACTN3 test does not guarantee a person's ability to be an elite athlete. The testing of young athletes according to the *ACTN3* polymorphism alone is not sufficient for choosing a type of sports. Testing a combination of gene polymorphisms seems to be much more effective when combining, for example, the *ACTN3* with *ACE* polymorphisms or adding some other candidate gene polymorphisms to the combination.

Our results concur with the results of other authors who concluded that the effect of the *ACE* and *ACTN3* gene variants was different for male and female athletes (2, 16). According to the phenotypic physical development indexes, athletes with the *ACE* I/I and *ACTN3* X/X genotype seem to have better results of physical fitness test (STEMP and handgrip strength) than the athletes with the *ACE* D/D and *ACTN3* R/R genotype. Our results showed that high handgrip strength and STEMP were dependent on both environmental (training, sex, age, etc.) and genetic factors like the *ACE* and *ACTN3* polymorphisms. The Lithuanian athletes with the *ACE* I/I and I/D as well as *ACTN3* X/X and R/X genotype have the potential to achieve higher results in sports categories requiring speed and power; therefore, the analyzed polymorphisms of these genes can be used as the criteria for the sport type selection.

The study groups used in this research were not large enough due to the limitations imposed by a small number of elite athletes available for study in each sports discipline. Regardless of a relatively small number of participants, our study groups demonstrate unique and clearly distinctive phenotypes. Each group contains elite-level athletes from a well-defined sports category that has known major determinants for success. Although in such small samples true positive associations could be masked, we believe that this paper gives valuable insights into the physical development of the athletes and the *ACE* and *ACTN3* genes.

### Conclusions

The *ACE* I and *ACTN3* X alleles determine speed and power for Lithuanian athletes. In line with other researchers, it can be confirmed that the absence of a functional ACTN3 in fast-twitch muscle fibers is compensated. Lithuanian athletes who are carriers of the *ACE* I/I and I/D as well as *ACTN3* X/X and R/X genotypes have the potential to achieve better results in power-requiring sports; therefore, the analyzed polymorphisms of these genes might be used as the criteria for the sport type selection.

### Statement of Conflict of Interest

The authors state no conflict of interest.

## *ACE* ir *ACTN3* genetinių variantų reikšmė Lietuvos didelio meistriškumo sportininkų raumenų funkcinėms savybėms

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**Raktažodžiai:** *ACE*, *ACTN3*, fiziologiniai rodikliai, fizinis pajėgumas.

**Santrauka**. *Įvadas.* Daugelio įvairių mokslinių tyrimų pagrindu angiotenziną konvertuojančio fermento (*ACE*) ir α-aktinino-3 (*ACTN3*) genai vertinami kaip stiprūs genai kandidatai, lemiantys žmogaus fizinį pajėgumą. Tačiau *ACE* I/D ir *ACTN3* R/X polimorfizmų sąsajos su fizinio pajėgumo fenotipu tyrimų, atliktų įvairiose populiacijose, duomenys buvo prieštaringi. Šio tyrimo tikslas – įvertinti *ACE* ir *ACTN3* genetinių variantų reikšmę Lietuvos sportininkų raumenų jėgos fenotipui.

*Tirtųjų kontingentas ir tyrimo metodai.* Ištirti 193 Lietuvos didelio meistriškumo sportininkai ir 250 bendrosios populiacijos asmenų (kontrolė). Genotipavimas buvo atliktas polimerazės grandininės reakcijos ir (arba) restrikcijos fragmentų ilgio polimorfizmų analizės metodu. Išmatuoti sportininkų antropometriniai duomenys ir raumenų jėga (plaštakų suspaudimo jėga ir vertikalus šuolis).

*Rezultatai.* Nustatyta, kad *ACE* I/I ir I/D genotipų dažniai tarp sportininkų didesni nei bendrojoje Lietuvos populiacijoje. *ACTN3* X/X ir *ACE* I/I genotipo Lietuvos sportininkų plaštakų jėga ir vertikalaus šuolio testo rezultatai aukštesni palyginus su *ACE* D/D ir *ACTN3* R/R genotipo sportininkais.

*Išvados.* Remiantis šiais rezultatais, daroma išvada, kad *ACE* I ir *ACTN3* X aleliai lemia greičio ir jėgos savybes. Mūsų atlikto tyrimo duomenis patvirtino analogiški duomenys kitų mokslininkų, kurie nustatė, kad *ACTN3* trūkumas greitai susitraukiančiose raumenų skaidulose yra kompensuojamas. *ACE* I/I ir I/D, *ACTN3* X/X ir R/X genotipo Lietuvos sportininkai, tikėtina, gali pasiekti aukštesnių sportinių rezultatų greičio ir jėgos sporto šakose. Nagrinėtus polimorfizmus galima panaudoti kaip kriterijus atrenkant sportininkus.

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