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Association analysis of *ACE, ACTN3* and *PPARGC1A* gene polymorphisms in two cohorts of European strength and power athletes

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ABSTRACT: The performance of professional strength and power athletes is influenced, at least partly, by genetic components. The main aim of this study was to investigate individually and in combination the association of *ACE* (I/D), *ACTN3* (R577X) and *PPARGC1A* (Gly482Ser) gene polymorphisms with strength/power-oriented athletes' status in two cohorts of European athletes. A cohort of European Caucasians from Russia and Lithuania (161 athletes: by groups – weightlifters (87), powerlifters (60), throwers (14); by elite status – 'elite' (104), 'sub-elite' (57); and 1,202 controls) were genotyped for *ACE*, *ACTN3* and *PPARGC1A* polymorphisms. Genotyping was performed by polymerase chain reaction and/or restriction fragment length polymorphism analysis. Statistically significant differences in *ACTN3* (R577X) allele/genotype distribution were not observed in the whole cohort of athletes or between analysed groups separately when compared with controls. The odds ratio for athletes compared to controls of the *ACE* I/I genotype was 1.71 (95% CI 1.01-2.92) in the Russian cohort and for the *ACE* I/D genotype it was 2.35 (95% CI 1.10-5.06) in the Lithuanian cohort. The odds ratio of being a powerlifter in *PPARGC1A* Ser/Ser genotype carriers was 2.11 (95% CI: 1.09-4.09, P = 0.026). The *ACTN3* (R577X) polymorphism is not associated with strength/power athletic status in two cohorts of European athletes. The *ACE* I/I genotype is probably the 'preferable genotype' for Russian athletes and the *ACE* I/D genotype for Lithuanian strength/power athletes. We found that the *PPARGC1A* (Gly482Ser) polymorphism is associated with strength/power athlete status. Specifically, the *PPARGC1A* Ser/Ser genotype is more favourable for powerlifters compared to controls.

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INTRODUCTION

Environmental and genetic factors influence muscle function, resulting in large variations in physical performance phenotype between individuals [1-2]. It is important to note that each genetic variant (polymorphism) can explain only a small proportion of variance in athletic phenotype. Therefore, various methodological approaches have been introduced to find an association between gene polymorphism and athlete performance [3]. Case-control association studies rely on the supposition that one allele of a candidate gene is more or less common in a group of elite athletes (case) than it is in the general population (control). Cross-sectional association studies examine whether athletes with a specific genotype/allele show different measures of a phenotypic trait compared to the rest of the athletes [3]. Another approach is genome-wide association studies that examine genetic markers at the whole genome level, to enable DNA markers to be linked with particular physical performance phenotypes [3-4].

The heritability of muscle strength and power has been shown to range from approximately 30% to 80% in various phenotypes such as handgrip strength, isometric knee strength, and elbow flexion [2]. The explosive and high-intensity nature of strength/power athletes results in a number of functional adaptations of the musculoskeletal and cardiovascular systems [5]. Strength/power athletes such as powerlifters, weightlifters and throwers, undertaking an anaerobic type of strength training, contain predominantly IIA and IIX muscle fibres [6-7]. Multiple genetic variants are thought to influence muscle function and physical performance phenotypes. Yet how the relevant polymorphisms influence muscle strength in power-orientated athletes (powerlifters, weightlifters and throwers) is unclear.

We focused on three common gene polymorphisms to explain individual variations in strength and power sports performance: (*1*) the I/D polymorphism (287-bp Alu-sequence insertion/deletion; SNP ID: rs1799752; 17q22-q24) of the angiotensin converting enzyme (*ACE*) gene; (2) the R577X polymorphism (C T transition at position 1747 in exon 16; SNP ID: rs1815739; 11q13-q14) of the alpha-actinin-3 (*ACTN3*) gene; (3) the G/A polymorphism (G A transition at position 1444 in exon 8, p.Gly482Ser, SNP ID: rs8192678; 4p15.1) of the peroxisome proliferator-activated receptor gamma coactivator-1 (*PPARGC1A*) gene [1-2, 8-9].

The *ACE* (I/D) and *ACTN3* (R577X) polymorphisms are two of the most studied physical performance gene variants and both have been associated with strength as well as other power phenotypes and elite athletic performance [1-2, 9]. The protein encoded by the human *ACE* gene is the most important component of the renin-angiotensin system [9-10]. Reported effects of *ACE* (I/D) polymorphism vary across studies and populations [9-11]. The *ACE* I allele initiates lower ACE enzyme activity and is associated with enhanced endurance performance. Several studies have shown that the *ACE* D allele is associated with greater strength and muscle volumes at baseline and an increased percentage of fast-twitch muscle fibres. In addition, the *ACE* D allele was associated with elite power athlete status [1, 10]. According to Eider et al., the D allele can be considered as an advantageous factor for athletes undergoing a heavy resistance training programme, developing strength abilities (such as powerlifters and weightlifters) [12-13].

The *ACTN3* gene encodes the protein alpha-actinin-3, a sarcomeric protein that is expressed in fast, type II fibres, where it plays an important role in the generation of explosive and powerful muscle contractions. The *ACTN3* nonsense (R577X) polymorphism is a strong candidate to influence elite athletic performance [9, 13, 14-16]. Alpha-actinin-3 deficiency (the XX genotype) reduces strength, muscle mass, and fast-twitch fibre diameter, but increases the proportion of slow-twitch muscle fibres [17-18]. Several case-control association studies have reported that the *ACTN3* RR genotype is over-represented or the *ACTN3* XX genotype is under-represented in strength and power athletes in comparison with controls [14-16]. Vincent et al. found that the number of type IIX fibres of the vastus lateralis was greater in the *ACTN3* RR genotype than the XX genotype group of young healthy men [18]. Furthermore, the hypothesis that the *ACTN3* R allele may confer some advantage in power performance events has been supported by several cross-sectional studies in elite athletes and non-athletes as well as mouse models of *ACTN3* deficiency [1, 12-16].

The *PPARGC1A* gene has also been the subject of several studies looking for associations between genotypes and athletic performance [1,19-21]. The peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α, encoded by *PPARGC1A*), a transcriptional coactivator of the PPAR family, is involved in mitochondrial biogenesis, fatty acid oxidation, glucose utilization, thermogenesis and angiogenesis [8, 19-21]. PGC-1α is one of the key regulators of skeletal muscle metabolism and coordinates the function of the genes involved in adaptation to physical performance [1, 20-21]. The expression of the *PPARGC1A* gene is related to both short-term exercise and endurance training in rodent models and humans [19]. Among all discovered variations in the *PPARGC1A* gene, the Gly482Ser polymorphism is of special interest [1, 8, 20-21]. The minor 482Ser allele of the *PPARGC1A* polymorphism is associated with reduced expression of *PPARGC1A*. It is hypothesized that the Gly482 allele of *PPARGC1A* is associated with a higher aerobic capacity [8, 20-21].

Based on knowledge about the role of *ACE* (I/D), *ACTN3* (R577X) and *PPARGC1A* (Gly482Ser) polymorphisms in skeletal muscle function, we hypothesize that a combination of these polymorphisms could also improve strength and power ability. Consequently, the aim of the study was to investigate individually and in combination the association of *ACE* (I/D), *ACTN3* (R577X) and *PPARGC1A* (Gly482Ser) gene polymorphisms with status of strength/power-oriented athletes (powerlifters, weightlifters and throwers) in two cohorts of European athletes.

MATERIALS AND METHODS

All procedures followed in this study meet the ethical standards in Sport and Exercise Science Research approved by appropriate local Ethics Committees and written informed consent was obtained from each participant.

This study was performed in a group of 114 Russian and 47 Lithuanian (aged 23.0 ± 6.5 years; male n = 128 and female n = 33) professional strength/power athletes (weightlifters (n = 87), powerlifters (n = 60), and throwers (n = 14)). All athletes were ranked in the top 10 nationally in respective sport discipline and grouped as being either 'elite-level' (n = 104, participated in international competitions) or 'sub-elite' (n = 57, participated only in national competitions) based on their best personal performance. The athletes were only included if they had never tested positive in anti-doping controls.

Controls were 1,202 (aged 29.0 ± 8.5 years; 540 males and 662 females) healthy, unrelated citizens of Russia (n = 947) and Lithuania (n = 255) without any competitive sport experience. All participants (athletes and controls) were Caucasians.

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes of the Lithuanian participants using a standard phenol-chloroform extraction method. The DNA was extracted from the buccal cells donated by the Russian subjects using DNKsorbA and ProbaGS sorbent kits (Central Research Institute of Epidemiology and DNA Technology, Russia) according to the manufacturers' instructions.

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., using the following primers: forward, 5'-CTGGAGACCACTCCCATCCTTTCT-3' and reverse, 5'-GATGTGGCCATCACATTCGTCAGAT-3' [22]. This method yields PCR fragments of

190 bp and 477 bp in the presence of the D and I alleles, respectively. Reaction products were visualized by electrophoresis on a 2% agarose gel and identified by ethidium bromide staining.

Genotyping of the *ACTN3* (rs1815739) and *PPARGC1A* (rs8192678) polymorphisms was performed using PCR. The resulting PCR products were genotyped by restriction fragment length polymorphism (RFLP). The *ACTN3* (R577X) polymorphism was amplified using the following primers: forward, 5'-CTGTTGCCTGTGGTAAGTGGG-3' and reverse, 5'-TGGTCACAGTATGCAGGAGGG-3'. The amplified fragment subsequently underwent digestion by DdeI restriction endonuclease (Thermo Fisher Scientific, Lithuania; SibEnzyme, Russia), described by Mills and colleagues [23]. Digested PCR fragments (108 bp, 97 bp and 86 bp fragments for the R allele; 205 bp and 86 bp for the X allele) were separated by 8% polyacrylamide gel electrophoresis.

The *PPARGC1A* polymorphism was amplified using PCR primers as follows: forward 5'-TTGTTCTTCCACAGATTCAGAC-3' and reverse 5'-GAAAAGGACCTTGAACGAGAG-3'. The analysed PCR product was cut by *MspI* restriction endonuclease (Thermo Fisher Scientific, Lithuania; SibEnzyme, Russia). Digested PCR fragments (449 bp fragment for the G allele; 274 bp and 175 bp for the A allele) were separated by 2% agarose gel [24].

The PCR results were scored by two independent investigators who were blind to subject data. No intra-observer variability was found on repeated readings of the same gel. The results of the genotyping were in agreement across the Russian and Lithuanian laboratories.

TABLE 1. Genotype and allele frequencies of *ACE* (I/D), *ACTN3* (R577X) and *PPARGC1A* (Gly482Ser) polymorphisms for the athletes' groups and controls from Russia and Lithuania.

Genotype	Russian cohort				Lithuanian cohort			
	Controls (n = 947)		Athletes (n = 114)		Controls (n = 255)		Athletes (n = 47)	
	Freq	%	Freq	%	Freq	%	Freq	%
ACE								
I/I	235	24.8	38	33.3	63	24.7	11	23.4
I/D	444	46.9	50	43.9	94	36.9	25	53.2
D/D	268	28.3	26	22.8	98	38.4	11	23.4
MAF		51.7		44.7		56.9		50
HWE p-value	0.06		0.23		0.0007		0.66	
ACTN3								
R/R	344	36.3	49	43	102	40	18	38.3
R/X	475	50.2	52	45.6	127	49.8	24	51.1
X/X	128	13.5	13	11.4	26	10.2	5	10.6
MAF		38.6		34.2		35.1		36.2
HWE p-value	0.07		0.89		0.14		0.47	
PPARGC1A								
Gly/Gly	424	44.8	62	54.4	132	51.76	24	51.1
Gly/Ser	416	43.9	35	30.7	106	41.57	22	46.8
Ser/Ser	107	11.3	17	14.9	17	6.67	1	2.1
MAF		33.3		30.3		27.5		25.5
HWE p-value	0.75		0.004		0.49		0.11	

Note: HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency

Data analysis

Genotype frequencies of the athletes were tested for compatibility with Hardy-Weinberg equilibrium (HWE). A chi-square test was used to assess the fit of the observed genotype frequencies to the HWE. The homogeneity hypothesis for genotype and allele frequency differences between groups was assessed by chi-squared or Fisher's exact test as appropriate. The level of significance was set at 0.05. The statistical software package R v. 3.2.1 was used to obtain the results. A logistic regression analysis was employed for the calculation of the odds ratio for the interaction of analysed polymorphisms in strength/power athlete and in control subjects.

RESULTS

The results of the distribution of *ACE* (I/D), *ACTN3* (R577X) and *PPARGC1A* (Gly482Ser) variants in Russian and Lithuanian athletes versus controls are presented in Table 1. In both athlete and control groups, the genotype distributions were in agreement with the Hardy-Weinberg equilibrium (HWE, $P > 0.05$ in all groups tested separately), except *PPARGC1A* (Gly482Ser) ($P = 0.004$) for the Russian athlete group and *ACE* (I/D) polymorphisms ($P = 0.0007$) for Lithuanian controls. The observed deviation of *ACE* genotype from HWE in the Lithuanian control group has been noted and discussed previously [25].

Statistically significant differences in *ACE* (I/D) and *ACTN3* (R577X) allele/genotype distribution were not observed in the whole cohort of Russian and Lithuanian athletes (Table 1) or in each group separately, i.e. groups of powerlifters, weightlifters and throwers when compared with controls (Table 2). However, the *ACE* I allele was more frequent in the athlete group compared to the controls (53.7% vs. 47.2%, P=0.08).

There were no significant differences in the *ACE* (I/D), *ACTN3* (R577X) or *PPARGC1A* (Gly482Ser) genotype or allele frequencies between males and females amongst both athletes and controls of both ethnic groups. Similarly, no significant differences were found between the subgroups of elite athletes and sub-elite athletes (data not shown).

Table 2 shows the association between genotype and strength/power athletic status for all participants. Statistically significant differences in genotype distribution were observed in the whole combined powerlifter group for the *PPARGC1A* polymorphism (Gly/Gly 62.3%, Gly/Ser 18%, Ser/Ser 19.7%) compared with weightlifters (Gly/Gly 47.7%, Gly/Ser 45.3%, Ser/Ser 7%; $P = 0.0009$) as well as with throwers (Gly/Gly 50%, Gly/Ser 50%, Ser/Ser 0%; $P = 0.019$) and with controls (Gly/Gly 46.3%, Gly/Ser 43.4%, Ser/Ser 10.3%, $P = 0.0002$).

TABLE 2. The *ACE* (I/D), *ACTN3* (R/X), *PPARGC1A* (Gly482Ser) genotype and allele frequency distributions amongst all participants.

Groups		Powerlifters	Weightlifters	Throwers	All athletes	Control group
Group size, n		61	86	14	161	1202
ACE						
Allele (%)	I	55.7	52.3	53.6	53.7	47.2
	D	44.3	47.7	46.4	46.3	52.8
P- value	compared to controls	0.191	0.633	0.355	0.08	-
Genotype (%)	I/I	21 (34.4)	23 (26.7)	5 (35.7)	49 (30.4)	298 (24.8)
	I/D	26 (42.6)	44 (51.2)	5 (35.7)	75 (46.6)	538 (44.8)
	D/D	14 (23)	19 (22.1)	4 (28.6)	37 (23)	366 (30.4)
P- value	compared to controls	0.194	0.628	0.255	0.102	-
ACTN3						
Allele (%)	R	68.8	61.0	75	65.2	62.1
	X	31.2	39.0	25	34.8	37.9
P- value	compared to controls	0.291	0.323	0.839	0.371	-
Genotype (%)	R/R	30 (49.2)	30 (34.9)	7 (50)	67 (41.6)	446 (37.1)
	R/X	24 (39.3)	45 (52.3)	7 (50)	76 (47.2)	602 (50.1)
	X/X	7 (11.5)	11 (12.8)	0	18 (11.2)	154 (12.8)
P- value	compared to controls	0.159	0.299	0.911	0.521	-
PPARGC1A						
Allele (%)	Gly	71.3	70.3	75	71.1	68.0
	Ser	28.7	29.7	25	28.9	32.0
P- value	compared to controls	0.585	0.575	0.647	0.199	-
Genotype (%)	Gly/Gly	38 (62.3)	41 (47.7)	7 (50)	86 (53.4)	556 (46.3)
	Gly/Ser	11 (18)	39 (45.3)	7 (50)	57 (35.4)	522 (43.4)
	Ser/Ser	12 (19.7)	6 (7)	0	18 (11.2)	124 (10.3)
P- value	compared to controls	0.0002*	0.444	0.610	0.149	-

Note: *P<0.05, statistically significant difference compared to control.
χ^2 =13.95, d.f. =1, p=0.0009 for *PPARGC1A* genotype frequencies in powerlifters versus weightlifters; χ^2 =7.89, d.f. =1, p=0.019 for *PPARGC1A* genotype frequencies in powerlifters versus throwers.

There were no significantly greater/lesser odds of harbouring any type of genotype when comparing the group of all athletes and controls. However, in the whole Russian cohort, the odds ratio of the genotype *ACE* I/I being a strength/power athlete was 1.71 (95% CI 1.01-2.92, P = 0.04) compared to controls. In the whole cohort of Lithuanian athletes, the odds ratio of the heterozygous *ACE* I/D genotype was 2.35 (95% CI 1.10-5.06, P = 0.028) compared to controls. It was revealed that the *ACE* I/I genotype is probably the 'preferable genotype' for Russian athletes and the *ACE* I/D genotype for Lithuanian strength/power athletes.

Nevertheless, after splitting the whole cohort of athletes according to sports discipline (without throwers due to an insufficient number of athletes for logistic regression), significant differences were determined between powerlifters and control groups. In the powerlifters as a whole, the odds ratio of the *PPARGC1A* Gly/Gly genotype was 1.92 (95% CI 1.13–3.26, P = 0.016) and that of Ser/Ser was 2.13 (95% CI 1.10–4.11, P = 0.0244) (Table 3).

Given that both *PPARGC1A* homozygous (Gly/Gly and Ser/Ser) genotypes are significant for powerlifter athletes in univariate analysis (Table 3), we performed combination analysis of homozygous *PPARGC1A* genotypes separately with *ACE* and *ACTN3* genotypes whose p-values in univariate analysis were lower than 0.10, i.e. *ACE* I/I and *ACTN3* R/R genotypes (Table 4). We found that in the analysed genotype combinations the only significant component was the *PPARGC1A* genotype: the odds ratio of *PPARGC1A* Ser/Ser genotype, OR=2.11 (95% CI: 1.09-4.09, P = 0.026), is higher compared to Gly/Gly genotype, OR=1.92 (95% CI: 1.13-3.26, P = 0.016) (Table 4). Hence the *PPARGC1A* Ser/Ser genotype is more favourable for powerlifter athletes compared to controls.

DISCUSSION

Human power and strength are typical quantitative and multifactorial traits that are influenced by both multiple genes and environmental factors [1-2]. The impact of individual gene variants and their

TABLE 3. Odds ratios for being weightlifters or powerlifters compared to controls in two cohorts from Russia and Lithuania

Gene	Geno-type	Weightlifters vs Controls				Powerlifters vs Controls			
		OR	95% CI		p-value	OR	95% CI		p-value
	D/D	0.65	0.38	1.09	0.104	0.68	0.37	1.25	0.215
ACE	I/D	1.30	0.83	2.00	0.250	0.92	0.55	1.54	0.743
	I/I	1.11	0.68	1.82	0.686	1.59	0.92	2.74	0.094
	R/R	0.91	0.57	1.44	0.680	1.64	0.98	2.75	0.060
ACTN3	R/X	1.09	0.71	1.70	0.688	0.65	0.38	1.09	0.104
	X/X	0.99	0.52	1.92	0.995	0.88	0.39	1.97	0.760
	Gly/Gly	1.06	0.68	1.64	0.799	1.92	1.13	3.26	0.016
PPARGC1A	Gly/Ser	1.08	0.70	1.68	0.729	0.29	0.15	0.56	0.0002
	Ser/Ser	0.65	0.28	1.53	0.324	2.13	1.10	4.11	0.024

TABLE 4. Genotype combinations for powerlifters compared to controls in the whole cohort from Russia and Lithuania.

	OR	95% CI		p-value
Combination I				
PPARGC1A Gly/Gly	1.92	1.13	3.26	0.016
ACTN3 R/R	1.63	0.97	2.74	0.063
ACE I/I	1.61	0.93	2.78	0.088
Combination II				
PPARGC1A Ser/Ser	2.11	1.09	4.09	0.026
ACTN3 R/R	1.63	0.97	2.74	0.068
ACE I/I	1.56	0.90	2.70	0.110

combination on athletic ability is at present a matter of investigation worldwide. Reports regarding the connection between the *ACE*, *ACTN3* and *PPARGC1A* polymorphisms and performance of strength/power-oriented athletes (powerlifters, weightlifters and throwers) are still limited. The present report is a genetic case-control association study. The novelty in the study is investigation of the *ACE* (I/D, rs1799752), *ACTN3* (R577X, rs1815739) and *PPARGC1A* (Gly482Ser, rs8192678) polymorphisms individually and in combination among strength/power athletes and non-athletic controls from Russia and Lithuania.

Our main findings were that 1) *ACE* (I/D) or *ACTN3* (R/X) genotype and allele frequency distributions are homogeneous comparing athletes with controls from Russia and Lithuania; 2) *ACE* I/I genotype is probably the 'preferable genotype' for Russian strength/power athletes and *ACE* I/D genotype for Lithuanian strength/power athletes. 3) An interesting finding was that when investigating *PPARGC1A* (Gly482Ser) polymorphism no significant results were obtained comparing the overall (Russian and Lithuanian) strength/power athlete group and controls. However, significant differences were determined between powerlifters and controls, i.e. the proportion of the *PPARGC1A* Gly/Gly and Ser/Ser genotype was higher for powerlifters. The calculated odds ratio indicated that the *PPARGC1A* Ser/Ser genotype is more favourable for powerlifter athletes.

To date, only *ACTN3* (R577X) has been associated with either endurance, strength or power performance with respect to the level of athletic performance, while *ACE* (I/D) is another highly studied candidate gene polymorphism with respect to elite athletic performance, providing less consistent results. A few reports have shown that the *ACTN3* R/R and R/X genotypes are associated with predisposition to power sports and positively correlated with elite strength/power athletes [15-16]. In this study, we found no significant association between the *ACTN3* (R577X) polymorphisms and strength/power performance in two cohorts from Russia and Lithuania. Our results are contrary to the hypothesis that the *ACTN3* (R577X) polymorphisms are associated with athletic strength and power ability (in groups of powerlifters, weightlifters and throwers).

An excess of the *ACE* I allele has been associated with some aspects of endurance performance and the D allele with sprint/power-related phenotypes. Several studies have reached quite opposite conclusions [1, 12]. The studies investigating the association between *ACE* (I/D) genotype and muscle size changes in response to strength training have demonstrated no association in muscles of the upper arm [10]. Several investigations have found no association of *ACE* (I/D) genotype with isometric and dynamic strength [10]. In the study by Eider, it was concluded that the D allele can be considered as an advantageous factor for strength athletes (such as powerlifters and weightlifters) but is not essential for developing sprint abilities in track-and-field athletes [12]. It is interesting that we failed to demonstrate an association of the *ACE* D allele with the status of weightlifters and powerlifters. We observed that the *ACE* I allele was more frequent in the whole cohort of athletes compared to the controls, though not significantly (P=0.08). Therefore, we performed an analysis for each cohort separately. It was found that the *ACE* I/I genotype is probably the 'preferable genotype' for Russian athletes

and the *ACE* I/D genotype for Lithuanian strength/power 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 (representing six ethnolinguistic groups of Lithuanians). The observed deviation of *ACE* genotype from HWE in the Lithuanian control group has been noted previously and discussed [25].

Consequently, we do not support the hypothesis that the *ACE* DD genotype is favourable for strength/power-oriented sports. Our findings are in keeping with several earlier studies showing an association of the I allele of the *ACE* gene with strength/power athlete status. Kim et al. reported that top level power-oriented athletes had a markedly diminished frequency of the DD genotype and the D allele than controls [26]. The same finding was reported by Shahmoradi et al. [27]. Wang et al. reported that short-distance swimmers have a prevalence of the *ACE* I allele in comparison with controls [28]. Furthermore, Pescatello et al. studied the associations among *ACE* I/D polymorphism and the response to a 12-week unilateral, upper-arm resistance training programme in the trained and untrained arms of 631 men and women. They reported greater improvements in maximal voluntary contraction (a measure of isometric strength) in the trained and untrained arms after training in carriers of the *ACE* I allele [10].

The *PPARGC1A* gene has also been the subject of several studies looking for associations between genotypes and physical performance phenotype [1, 8, 20-21]. The results of numerous studies suggest that the variation (Gly482Ser) for this gene is associated with human physical performance and significantly influences the response of athletes to physical activity. Lucia et al. were the first to test the hypothesis that the minor 482Ser allele of *PPARGC1A* is less frequent in elite endurance athletes. The authors reported that the 482Ser allele was less prevalent in a group of athletes compared with controls and suggested that the *PPARGC1A* Gly482 allele may be one of the genetic factors that predetermine aerobic capacity [21]. The association study conducted with Polish athletes revealed that the frequency of the *PPARGC1A* 482Ser allele was significantly lower in endurance, strength-endurance and sprint-strength athletes than in controls [8]. The authors suggested that the *PPARGC1A* Gly482 allele is important for every athlete regardless of the type of exercise [8].

In this study analysing Russian and Lithuanian athletes together, we observed significant differences between the *PPARGC1A* genotype distributions for the powerlifter group and controls ($P = 0.0002$). Furthermore, the *PPARGC1A* Gly/Gly and Ser/Ser genotypes were over-represented in powerlifter athletes compared to controls. An interesting finding was that the *PPARGC1A* Ser/Ser genotype is more favourable for powerlifter athletes compared to the *PPARGC1A* Gly/Gly genotype.

The physiological explanation for these investigations is the role of the *PPARGC1A* gene (encoding the protein PPARG coactivator-1α (PGC-1α)) in energy metabolism, mitochondrial regulation and biogenesis. PGC-1α is a powerful transcriptional coactivator of numerous genes [8, 20]. PGC-1α function in muscle and other tissues is important for the optimization of whole body metabolism. The expression of *PPARGC1A* in skeletal muscle is functional in both short-term exercise and endurance training in rodent models and human subjects [1, 19, 29]. There is strong evidence that the Gly482Ser polymorphism in the *PPARGC1A* gene is associated with skeletal muscle fibre-type conversion (i.e. from glycolytic type IIb to the more mitochondria-rich types IIa and I, which utilize oxidative metabolism) [8, 21]. Since muscle represents a large portion of body mass, changes in mitochondrial content have a significant impact on whole body metabolism [19, 29]. Strength/power athletes' performance is determined by the combination of both peak force/power and the ability to sustain and repeat high-intensity efforts for extended periods during a competition [5, 30]. A main deciding factor of maximal sustainable power is the mitochondrial amount in the recruited muscle fibres. Ideally, an athlete strives to maximize both muscle power and endurance [30].

Nevertheless, the functional impact of the *PPARGC1A* (Gly482Ser) polymorphism on strength and power capacity remains unclear. Further investigations are required to clarify the effect of candidate gene variants on strength/power performance phenotype. There are already other genetic variants that have been reported to show associations with power and strength athlete status [1-2], and we strongly suspect that many additional common polymorphisms, and probably rare mutations as well, will be shown to be associated with strength/power phenotypes in due course.

The study groups used in this research were not large enough, due to limitations imposed by the small number of elite athletes available for study in each sports discipline. However, considering that there is a limited number of elite strength/power athletes (weightlifters, powerlifters, and throwers) worldwide and also in Russia and Lithuania, we had no other resources to gather additional samples for this study.

Notwithstanding, we believe that the results of our study are valid overall. The cases (athletes) clearly presented the main study phenotype (i.e. being an elite or sub-elite athlete). We studied some of the best elite strength/power athletes (weightlifters, powerlifters and throwers). Genetic assessment was accurate and unbiased.

CONCLUSIONS

In conclusion, no association was found between the *ACTN3* (R577X) polymorphisms and elite strength/power athletic status in two cohorts of European athletes. The *ACE* I/I genotype is probably the 'preferable genotype' for Russian athletes and the *ACE* I/D genotype for Lithuanian strength/power athletes. We found that the *PPARGC1A* (Gly482Ser) polymorphism is associated with strength/power athlete status. Specifically, the *PPARGC1A* Ser/Ser genotype is more favourable for powerlifter athletes compared to controls.

In general, our results indicate that the *PPARGC1A* Ser/Ser genotype

benefits powerlifter athletes, who are characterized by an increased contribution of the anaerobic system to the production of energy required for exercise. One might speculate that the *PPARGC1A* 482Ser allele is an advantageous factor for athletes undergoing a heavy resistance training programme, developing strength abilities (such as powerlifters). This finding supports the general assumption that the Ser/Ser genotype of the *PPARGC1A* gene is beneficial for sports disciplines characterized by predominantly anaerobic energy production and is related to greater power performance.

Our data confirm the hypothesis that the physical performance phenotype is inherited by a number of candidate genes, and each of them, taken separately, lowly or insignificantly contributes to the overall development of human physical performance traits.

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REFERENCES

1. Ahmetov II, Fedotovskaya ON. Current Progress in Sports Genomics. Adv Clin Chem. 2015;70:247-314.
2. Hughes DC, Day SH, Ahmetov II, Williams AG. Genetics of muscle strength and power: Polygenic profile similarity limits skeletal muscle performance. J Sports Sci. 2011;29(13):1425-1434.
3. Sawczuk M, Maciejewska-Karłowska A, Cięszczyk P, Leońska-Duniec A. IS GNB3 C825T polymorphism associated with elite status of Polish athletes? Biol Sport. 2014;31:21-25.
4. Ahmetov II, Kulemin NA, Popov DV, Naumov VA, Akimov EB, Bravy YR, Egorova ES, Galeeva AA, Generozov EV, Kostryukova ES, Larin AK, Mustafina LJ, Ospanova EA, Pavlenko AV, Starnes LM, Żmijewski P, Alexeev DG, Vinogradova OL, Govorun VM. Genome-wide association study identifies three novel genetic markers associated with elite endurance performance. Biol Sport. 2015;32(1):3-9.
5. Storey A, Smith HK. Unique Aspects of Competitive Weightlifting: Performance, Training and Physiology. Sports Med. 2012;42(9):769-790.
6. Zawadowska B, Majerczak J, Semik D, Karasinski J, Kolodziejski L, Kilarski WM, Duda K, Zoladz JA. Characteristics of myosin profile in human vastus lateralis muscle in relation to training background. Folia Histochem Cytobiol. 2004;42(3):181-190.
7. Ahmetov II, Vinogradova OL, Williams AG. Gene Polymorphisms and Fiber-Type Composition of Human Skeletal Muscle. Int J Sport Nutr Exerc Metabol. 2012;22:292-303.
8. Maciejewska A, Sawczuk M, Cieszcyk P, Mozhayskaya I, Ahmetov II. The PPARGC1A gene Gly482Ser in Polish and Russian athletes. J Sports Sci. 2012;30(1):101-113.
9. Scott RA, Irving R, Irwin L, Morrison E, Charlton V, Austin K, Tladi D, Deason M, Headley SA, Kolkhorst FW, Yang N, North K, Pitsiladis YP. ACTN3 and ACE Genotypes in Elite Jamaican and US Sprinters. Med Sci Sports Exerc. 2010;42(1):107-112.
10. Pescatello LS, Kostek MA, Gordish-Dressman H, Thompson PD, Seip RL, Price TB, Angelopoulos TJ, Clarkson PM, Gordon PM, Moyna NM, Visich PS, Zoeller RF, Devaney JM, Hoffman EP. ACE ID genotype and the muscle strength and size response to unilateral resistance training. Med Sci Sports Exerc. 2006;38:1074-1081.
11. Drozdovska SB, Dosenko VE, Ahmetov II, Ilyin VN. The association of gene polymorphisms with athlete status in Ukrainians. Biol Sport. 2013;30:163-167.
12. Eider J, Cieszczyk P, Ficek K, Leonska-Duniec A, Sawczuk M, Maciejewska-Karlowska A, Zarebska A. The association between D allele of the ACE gene and power performance in Polish elite atletes. Sci Sports. 2013;28(6):325-330.
13. McCauley T, Mastana S, Hossack J, MacDonald M, Folland J. ACE I/D and ACTN3 R577X genotypes and human muscle functional and contractile properties. Exp Physiol. 2008;94.1:81-89.
14. Eynon N, Ruiz JR, Femia P, Pushkarev VP, Cieszczyk P, Maciejewska-Karlowska A, Sawczuk M, Dyatlov DA, Lekontsev EV, Kulikov LM, Birk R, Bishop DJ, Lucia A. The ACTN3 R577X Polymorphism across Three Groups of Elite Male European Athletes. PLoS One. 2012;7(8):e43132.
15. Cieszczyk P, Sawczuk M, Maciejewska-Karlowska A, Ficek K. ACTN3 R577X polymorphism in top-level Polish rowers. J Exerc Sci Fit. 2012;10(1):12-15.
16. Yang N, MacArthur DG, Gulbin JP, Hahn AG, Beggs AH, Easteal S, North K. ACTN3 genotype is associated with human elite athletic performance. Am J Hum Genet. 2003;73:627-631.
17. Ahmetov II, Druzhevskaya AM, Lyubaeva EV, Popov DV, Vinogradova OL, Williams AG. The dependence of preferred competitive racing distance on muscle fibre type composition and ACTN3 genotype in speed skaters. Exp Physiol. 2011;96(12):1302-1310.
18. Vincent B, De Bock K, Ramaekers M, Van den Eede E, Van Leemputte M, Hespel P, Thomis MA. ACTN3 (R577X) genotype is associated with fiber type distribution. Physiol Genomics. 2007;32:58-63.
19. Liang H, Walter F. PGC-1a: a key regulator of energy metabolism. Adv Physiol Educ. 2006;30:145-151.
20. Ahmetov II, Rogozkin VA. The Role of PGC-1 in the Regulation of Skeletal Muscle Metabolism. Hum Physiol. 2013;39(4):441-449.
21. Lucia A, Gomez-Gallego F, Barroso I, Rabadan M, Bandres F, San Juan AF, Chicharro JL, Ekelund U, Brage S, Earnest CP, Wareham NJ, Franks PW. PPARGC1A genotype (Gly482Ser) predicts exceptional endurance capacity in European men. J Appl Physiol. 2005;99:344-348.
22. Rigat B, Hubert C, Corvol P, Soubrier F. PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCP1) (dipeptidylcarboxypeptidase1). Nucl Acids Res. 1992;20:1433.
23. Mills MA, Yang N, Weinberger RP, Vander Woude DL, Beggs AH, Easteal S, North K. Differential expression of the actin-binding proteins, -actinin-2 and -3, in different species: implications for the evolution of functional redundancy. Hum Mol Genet. 2001;10:1335-1346.
24. Chen S, Yan W, Huang J, Yang W, Gu D. Peroxisome proliferator-activated receptor-gamma coactivator-1alpha polymorphism is not associated with essential hypertension and type 2 diabetes mellitus in Chinese population. Hypertens. 2004;27:813-820.
25. Gineviciene V, Pranckeviciene E, Milasius K, Kucinskas V. Gene variants related to the power performance of the Lithuanian athletes. Cent Eur J Biol. 2011;6:48-57.
26. Kim CH, Cho JY, Jeon JY, Koh YG, Kim YM, Kim HJ, Park M, Um HS, Kim C. ACE DD genotype is unfavorable to Korean short-term muscle power athletes. Int J Sports Med. 2010;31(1):65-71.

27. Shahmoradi S, Ahmadalipour A, Salehi M. Evaluation of ACE gene I/D polymorphism in Iranian elite atletes. Adv Biomed Res. 2014;3:207.
28. Wang G, Mikami E, Chiu LL, De Perini A, Deason M, Fuku N, Miyachi M, Kaneoka K, Murakami H, Tanaka M, Hsieh LL, Hsieh SS, Caporossi D, Pigozzi F, Hilley A, Lee R, Galloway SDR, Gulbin J, Rogozkin VA, Ahmetov II, Yang N, North KN, Ploutarhos S, Montgomery HE, Bailey MES, Pitsiladis YP. Association analysis of ACE and ACTN3 in Elite Caucasian and East Asian Swimmers. Med Sci Sports Exerc. 2013;45:892-900.
29. Uguccioni G, D'souza D, Hood DA. Regulation of PPAR Coactivator-1 Function and Expression inMuscle: Effect of Exercise. PPAR Res. 2010; ID 937123, doi: 10.1155/2010/937123.
30. Meijer JP, Jaspers RT, Rittweger J, Seynnes OR, Kamandulis S, Brazaitis M, Skurvydas A, Pisot R, Simunic B, Narici MV, Degens H. Single muscle fibre contractile properties differ between bodybuilders, power athletes and controls. Exp Physiol. 2015;20. doi: 10.1113/EP085267. [Epub ahead of print].