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THE ASSOCIATION OF GENE POLYMORPHISMS WITH ATHLETE STATUS IN UKRAINIANS

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ABSTRACT: Athletic performance is a polygenic trait influenced by both environmental and genetic factors. Objective: to investigate individually and in combination the association of common gene polymorphisms with athlete status in Ukrainians. Methods: A total of 210 elite Ukrainian athletes (100 endurance-oriented and 110 power-oriented athletes) and 326 controls were genotyped for ACE I/D, HIF1A Pro582Ser, NOS3 -786 T/C, PPARA intron 7 G/C, PPARG Pro12Ala and PPARGC1B Ala203Pro gene polymorphisms, most of which were previously reported to be associated with athlete status or related intermediate phenotypes in different populations. Results: Power-oriented athletes exhibited an increased frequency of the HIF1A Ser (16.1 vs. 9.4%, $P = 0.034$) and NOS3 T alleles (78.3 vs. 66.2%, $P = 0.0019$) in comparison with controls. Additionally, we found that the frequency of the PPARG Ala allele was significantly higher in power-oriented athletes compared with the endurance-oriented athletes (24.7 vs. 13.5%; $P = 0.0076$). Next, we determined the total genotype score (TGS, from the accumulated combination of the three polymorphisms, with a maximum value of 100 for the theoretically optimal polygenic score) in athletes and controls. The mean TGS was significantly higher in power-oriented athletes (39.1 ± 2.3 vs. 32.6 ± 1.5 ; $P = 0.0142$) than in controls. Conclusions: We found that the HIF1A Ser, NOS3 T and PPARG Ala alleles were associated with power athlete status in Ukrainians.

KEY WORDS: gene polymorphisms, genetic markers, sport selection, athletic performance.

INTRODUCTION

Genetics has a great influence over components of athletic performance such as strength, power, endurance, muscle fibre size and composition, flexibility, neuromuscular coordination, temperament and other phenotypes. Accordingly, athlete status is a heritable trait: around 66% of the variance in athlete status is explained by additive genetic factors. The remaining variance is due to non-shared environmental factors [10]. Despite the relatively high heritability of athlete status, the search for genetic variants contributing to predisposition to success in certain types of sport has been a challenging task.

Sports genomics is a relatively new scientific discipline focusing on the organization and functioning of the genome of elite athletes. The era of sports genomics began in the early 2000s after deciphering the human DNA structure and discovery of the first genetic markers associated with athletic performance (e.g. ACE, ACTN3 and AMPD1 gene variations). With genotyping becoming widely available, a large number of genetic case-control studies evaluating candidate gene variants have been published with largely unconfirmed associations with elite athlete status.

A recent review provided evidence that at least 79 genetic markers (located within 40 autosomal genes, mitochondrial DNA and Y-chromosome) are linked to elite athlete status (59 endurance-related and 20 power/strength-related genetic markers) [2]. Although most of the case-control and association studies have not yet been replicated in independent samples, 20 genetic markers have shown positive associations with athlete status in at least two studies (14 endurance-related genetic markers: ACE I, ACTN3 577X, ADRB2 16Arg, AMPD1 Gln12, BDKRB2 -9, COL5A1 rs12722 T, GABPB1 rs7181866 G and rs12594956 A, HFE 63Asp, KCNJ11 Glu23, PPARA rs4253778 G, PPARG rs2016520 C, PPARGC1A Gly482, UCP3 rs1800849 T; and 6 power/strength-related genetic markers: ACE D, ACTN3 Arg577, AMPD1 Gln12, HIF1A 582Ser, NOS3 rs2070744 T, PPARA rs4253778 C).

The aim of the study was to investigate the association of ACE I/D, HIF1A Pro582Ser, NOS3 -786 T/C, PPARA intron 7 G/C, PPARG Pro12Ala and PPARGC1B Ala203Pro gene polymorphisms with athlete status in Ukrainians. Of the six gene variants, the HIF1A, PPARA, PPARG and PPARGC1B genes code for transcription factors

and coactivators (primarily involved in glucose, insulin and lipid metabolism, mitochondrial biogenesis, thermogenesis, and regulation of muscle fibre type composition), while *ACE* and *NOS3* code for enzymes involved in the regulation of vascular functions (Table 1).

MATERIALS AND METHODS

Study participants. In total, 210 Ukrainian athletes (145 males and 65 females; age 22.1 ± 2.5 years; 65 elite athletes, 127 sub-elite athletes and 18 non-elite athletes) were recruited from the endurance-oriented ($n=100$; cross-country skiers, rowers) and power-oriented ($n=110$; short distance runners, short distance swimmers, jumpers, throwers) sports. Controls were 326 healthy unrelated Ukrainians (187 males and 139 females; age 14-54 years) without any competitive sport experience. The athletes and control groups were all Caucasians.

The Regional Ethics Committee (Kiev, Ukraine) approved the study and written informed consent was obtained from each participant. All experiments were performed in accordance with the ethical standards of the Helsinki Declaration.

Genotyping

Molecular genetic analysis was performed with DNA samples obtained from epithelial mouth cells using a Diatom™ DNA Prep kit according to the manufacturer's instructions (Biokom, Russia). Genotyping for 6 gene polymorphisms was performed by PCR and restriction enzyme digestion as reported previously [6]. All genotyping analyses were conducted blind to subject identity.

Calculation of total genotype score

To quantify the combined influence of polymorphisms associated with power or endurance phenotypes, an algorithm [16,24] was used to incorporate all favourable genotype scores for any given in-

dividual in a simple additive model. The total score was then transformed mathematically to lie within the range 0–100 and labelled the 'total genotype score' (TGS). A TGS of 100 represents a 'perfect' polygenic profile for power/endurance and a TGS of 0 represents the 'worst' possible profile for power/endurance.

Statistical analysis

Statistical analyses were conducted using SPSS ver. 17.0 software package. Genotype distribution and allele frequencies between each of the two groups of athletes (endurance-oriented and power-oriented) and controls were compared using χ^2 tests. *P* values < 0.05 were considered statistically significant.

RESULTS

Genotype distributions of 6 gene polymorphisms in the control group and amongst all athletes were in Hardy-Weinberg equilibrium. There were no significant differences in *ACE*, *PPARA* and *PPARGC1B* genotype and allele frequencies between different groups of athletes and controls (Table 2). Power-oriented athletes exhibited an increased frequency of the *HIF1A* Ser (16.1 vs. 9.4%, *P* = 0.034) and *NOS3* T alleles (78.3 vs. 66.2%, *P* = 0.0019) in comparison with controls (Table 2). Compared with the *HIF1A* Pro/Pro carriers, the odds ratio (OR) of being a power-oriented athlete in Ser allele carriers (Pro/Ser+Ser/Ser) was 2.042 (95% confidence interval (CI): 1.078-3.871, *P* = 0.0315). Similarly, the OR of being a power-oriented athlete in the *NOS3* TT genotype carriers was 2.262 (95% CI: 1.396-3.664; *P* = 0.008).

Additionally, we found that the frequency of the *PPARG* Ala allele was significantly higher in power-oriented athletes compared with the endurance-oriented athletes (24.7 vs. 13.5%; *P* = 0.0076). Furthermore, the distribution of *PPARG* genotypes was significantly different between endurance-oriented athletes and controls (*P* = 0.012).

TABLE 1. CANDIDATE GENES FOR ATHLETE STATUS; THEIR FULL NAMES, FUNCTIONS OF GENE PRODUCTS, ASSOCIATED PHENOTYPES AND INTERACTIONS

Gene	Full name	Functions, associated phenotypes and interactions
<i>ACE</i>	Angiotensin I converting enzyme	Regulates circulatory homeostasis through the synthesis of vasoconstrictor angiotensin II and the degradation of vasodilator kinins.
<i>HIF1A</i>	Hypoxia inducible factor 1, α subunit	Regulates the transcription of numerous genes in response to hypoxic stimuli. Genes responsive to HIF1 are involved in the processes of erythropoiesis, angiogenesis, and metabolism and include those encoding erythropoietin, VEGF, PPAR α and glycolytic enzymes.
<i>NOS3</i>	Nitric oxide synthase 3	Generates nitric oxide (NO) in blood vessels and is involved with regulating vascular function. NO is an important cellular signalling molecule involved in many physiological and pathological processes. It is a powerful vasodilator with a short half-life of a few seconds in the blood.
<i>PPARA</i>	Peroxisome proliferator-activated receptor α	Regulates liver, heart and skeletal muscle lipid metabolism, glucose homeostasis, mitochondrial biogenesis, cardiac hypertrophy, expression of <i>UCP2</i> and <i>UCP3</i> genes.
<i>PPARG</i>	Peroxisome proliferator-activated receptor γ	Plays a critical physiological role as a central transcriptional regulator of adipogenic and lipogenic programmes, insulin sensitivity and glucose homeostasis.
<i>PPARGC1B</i>	Peroxisome proliferator-activated receptor γ coactivator 1 β	Regulates fatty acid oxidation, mitochondrial biogenesis, formation of muscle fibres; co-activates PPAR α and PPAR γ .

TABLE 2. GENOTYPE DISTRIBUTIONS AND MINOR ALLELE FREQUENCIES OF CANDIDATE GENES IN UKRAINIAN ATHLETES AND CONTROLS

Gene	Polymorphism	Genotype / allele	Endurance-oriented athletes	Power-oriented athletes	Controls
<i>ACE</i>	Alu I/D		n=84	n=108	n=283
		II	26.5	25.9	25.1
		ID	48.2	45.4	53.0
		DD	25.3	28.7	21.9
		MAF (D)	0.494	0.514	0.484
<i>PPARA</i>	intron 7 G/C		n=80	n=71	n=85
		GG	73.8	73.2	67.1
		GC	26.0	23.9	30.6
		CC	0	2.8	2.7
		MAF (C)	0.130	0.140	0.180
<i>PPARG</i>	Pro12Ala		n=100	n=87	n=318
		Pro/Pro	77.0	55.2	64.2
		Pro/Ala	19.0	40.2	34.0
		Ala/Ala	4.0	4.6	1.8
		MAF (Ala)	0.135 [§]	0.247	0.189
<i>PPARGC1B</i>	Ala203Pro		n=73	n=59	n=81
		Ala/Ala	84.4	86.4	88.9
		Ala/Pro	13.7	11.9	11.1
		Pro/Pro	1.4	1.7	0
		MAF (Pro)	0.082	0.07	0.05
<i>HIF1A</i>	Pro582Ser		n=81	n=59	n=260
		Pro/Pro	86.4	69.5	82.3
		Pro/Ser	13.6	28.8	16.5
		Ser/Ser	0	1.7	1.2
		MAF (Ser)	0.07	0.161*	0.094
<i>NOS3</i>	-786 T/C		n=82	n=90	n=321
		TT	45.1	63.3	43.3
		TC	46.3	30.0	45.8
		CC	8.5	6.7	10.9
		MAF (C)	0.317 [§]	0.217*	0.338

Note: MAF, minor allele frequency. * $P < 0.05$, statistically significant differences between power-oriented athletes and controls. [§] $P < 0.05$, statistically significant differences between endurance-oriented athletes and power-oriented athletes.

Next, we determined the TGS in athletes and controls. The mean TGS was significantly higher in power-oriented athletes (39.1 ± 2.3 vs. 32.6 ± 1.5 ; $P = 0.0142$) than in controls.

DISCUSSION

The present study demonstrates that the *HIF1A* Pro582Ser, *NOS3* -786 T/C and *PPARG* Pro12Ala polymorphisms are associated with elite athlete status in Ukrainians. We found that the *HIF1A* Ser, *NOS3* T and *PPARG* Ala alleles were over-represented in power-oriented athletes compared with controls or endurance-oriented athletes. We also identified a polygenic profile (by the use of combination of the *HIF1A*, *NOS3* and *PPARG* polymorphisms) of power-oriented athletes that allowed us, at least partly, to distinguish power-oriented athletes from controls. These polymorphisms have already been associated with power athlete status and/or power-related phenotypes in other studies [3,4,9,13,14,15,20], so our data replicate those previous findings.

Of those genes listed, *HIF1A* codes for hypoxia-inducible factor 1 (HIF-1A), which controls the expression of several genes impli-

cated in various cellular functions including glucose metabolism (glucose transporters and glycolytic enzymes). A missense polymorphism, Pro582Ser, is present in exon 12 (C/T at bp 85; rs11549465). The rare T allele is predicted to result in a proline to serine change in the amino acid sequence of the protein. This substitution increases HIF-1A protein stability and transcriptional activity [21], and therefore may improve glucose metabolism and lower the risk of type 2 diabetes [19]. The frequency of the *HIF1A* Ser allele was significantly higher in Russian weightlifters and wrestlers than in controls and increased with their levels of achievement [3,14]. These results were replicated in a cohort of Polish power-orientated athletes [9], but not in Israeli sprinters [13]. Furthermore, the Ser allele was significantly associated with an increased proportion of fast-twitch muscle fibres in *m. vastus lateralis* of all-round speed skaters [3].

Nitric oxide (NO) is involved in human skeletal muscle uptake during exercise [18] and modulation of oxygen consumption in skeletal muscles [23]. Dietary nitrate supplementation enhances muscle contractile efficiency during knee-extensor exercise and tolerance to high-intensity exercise in humans [7,8]. We have previously shown

that the level of the endothelial nitric oxide synthase gene (*NOS3*) mRNA and *NOS3* (*NOS3* generates NO in blood vessels) activity in isolated platelets is higher in TT genotype carriers of the -786 T/C (rs2070744) polymorphism [12]. Therefore, one might anticipate that genetic variation in the *NOS3* gene could be associated with power/sprint performance. Indeed, Gómez-Gallego et al. [15] found that the frequency of the *NOS3* T allele was significantly higher in 53 Spanish elite power-oriented athletes (jumpers, throwers, sprinters) compared to 100 non-athletic controls (frequency of the T allele: 71.0% vs. 56.0%; $P = 0.015$). These results were confirmed in the second study involving Italian power-oriented athletes [20] and in the present study.

Peroxisome proliferator-activated receptor γ (PPAR γ ; encoded by *PPARG*) plays a critical physiological role as a central transcriptional regulator of adipogenic and lipogenic programmes, insulin sensitivity and glucose homeostasis. The Ala variant of the *PPARG* gene Pro12Ala polymorphism (rs1801282 C/G) is associated with decreased receptor activity [11], improved insulin sensitivity [11] and increased body mass index in humans [5,17]. Carriers of the Ala allele show a better glycaemic response to exercise training [1], higher rates of skeletal muscle glucose uptake [22] and greater cross-sectional area of muscle fibres [4]. In a study of Russian power-oriented athletes ($n = 260$), a higher frequency (23.8% vs. 15.1%, $P < 0.0001$) of the *PPARG* 12Ala allele compared to 1,073 controls was reported [4]. The present study involving Ukrainian athletes has confirmed those findings.

On the other hand, we could not confirm the association between the *ACE*, *PPARA* and *PPARGC1B* gene polymorphisms and athletic

performance, presumably due to the limited number of studied athletes. In addition, our study is limited to 6 common polymorphisms which were primarily selected because of previously reported associations with various aspects of power performance. There are already other genetic variants that have been reported to show associations with aspects of power [2,16], and we strongly suspect that many additional common polymorphisms, and probably rare mutations as well, will be shown to be associated with power performance or a power phenotype in due course.

CONCLUSIONS

In conclusion, we found that the *HIF1A* Ser, *NOS3* T and *PPARG* Ala alleles were associated with power athlete status in Ukrainians. Our data also suggest an overall more 'favourable' polygenic profile in power-oriented athletes compared to controls. The results are in agreement with previous studies and suggest an opportunity to use the analysis of *HIF1A* Pro582Ser, *NOS3* -786 T/C and *PPARG* Pro12Ala polymorphisms along with other gene variations and standard phenotypic assessment in sports selection.

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Conflict of interest

The authors report no conflicts of interest.

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