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THE *HIF1A* GENE PRO582SER POLYMORPHISM IN POLISH POWER-ORIENTATED ATHLETES

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ABSTRACT: In mammalian cells, oxygen homeostasis is regulated by the transcription factor hypoxia-inducible factor-1 (HIF 1). To date, despite the fact that *HIF1A* has been associated with athletic performance, only two reports have investigated the hypothesis that *HIF1A* Pro582Ser genotype distribution may differ for power-orientated athletes and controls.The aim of this study was to perform confirmation studies to analyse the possible importance of the *HIF1A* Pro582Ser polymorphism in power-orientated athletes. The study was carried out on power-orientated athletes (short distance runners, short distance swimmers and weightlifters) and sedentary individuals, representing possible relationships between genotype and physical performance. DNA was extracted from buccal cells donated by the subject. Genotyping was carried out by PCR. Significance was assessed by Chi square (χ^2) analysis.The results revealed that the frequency of the *HIF1A* Pro/Ser genotype (34.18% vs. 18.11%; $P = 0.01$) and Ser allele (17.09% vs. 9.05%; $P = 0.01$) was significantly higher in the power-orientated athletes compared to sedentary controls. The results suggest that the *HIF1A* gene can be taken into consideration as a genetic marker in power-orientated athletes. However, these conclusions should be supported with more experimental studies on *HIF1A* polymorphisms in elite athletes.

KEY WORDS: HIF1, gene polymorphism, power-orientated athletes

INTRODUCTION

Hypoxia is defined as inadequate oxygen supply to the cells and tissues of the body [9]. This state is typical in power-orientated sports, where oxygen deprivation (exercise-induced hypoxia) is observed in the muscular tissue with the intensification of the function of the neuromuscular system. The efficiency of energy supply to human skeletal muscle in the hypoxic state is dependent on many factors affecting the "high anaerobic potential" (i.e. high reserves of ATP, high concentration and activities of glycolysis and phosphagenic system enzymes, creatine phosphate, glycogen, etc.) [1].

In mammalian cells, oxygen homeostasis is regulated by the transcription factor hypoxia-inducible factor-1 (HIF-1) [14]. HIF-1 regulates inter alia the expression of genes providing cell adaptation for low oxygen tension (including genes involved in angiogenesis, glucose metabolism, glucose transport, vasomotor control and erythropoiesis), many of which are implicated in either the delivery of oxygen and nutrients to cells, or controlling cellular utilization of these substrates [3,12,13,15,16].

HIF-1 is expressed in all mammalian tissues, including skeletal muscle (at a higher level in fast glycolytic muscle fibres) [18]. HIF-1 is a dimeric protein composed of the regulatory subunit HIF-1A and the constitutively expressed subunit HIF-1B [15].

Under normoxic conditions HIF-1a undergoes hydroxylation at specific prolyl residues, which leads to an immediate ubiquitination and subsequent proteasomal degradation of the a subunit. Additionally, hydroxylation of an asparaginyl residue blocks the transcriptional activity of HIF-1 due to inhibition of its interaction with co-activators. In contrast, under hypoxic conditions, abolition of prolyl hydroxylation results in HIF-1A stabilization, whereas the lack of asparaginyl hydroxylation allows the transcriptional activity [19].

The Pro582Ser polymorphism (rs11549465) was detected in the *HIF1A* gene that encodes the α subunit of HIF-1 protein, resulting in the replacement of proline (Pro) with serine (Ser) at amino acid 582 [4]. It was reported that this rare Ser582 allele, rather than the wild-type Pro582 allele, was associated with increased transcription activity and stability of HIF1A protein, and hence increases the hypoxic resistance of cells (high glycolytic potential, high angiogenesis) [13]. These data suggest that *HIF1A* Ser allele carriers are more predisposed to power-orientated athletics than Pro/Pro homozygotes.

This conclusion seems to be additionally supported by Ahmetov et al. [2], who found that carriers of the Pro/Ser genotype had a significantly higher percentage of type IIX muscle fibres (fast-twitch glycolytic, which are used predominantly in high explosive events, such as the 100-m sprint) than those homozygous for the Pro582 allele.

The aim of this study was to analyse the possible importance of the *HIF1*A gene Pro582Ser polymorphism in Polish power-orientated athletes and sedentary individuals, representing the possible relationships with genotype and physical performance.

MATERIALS AND METHODS

Ethics Committee. The Pomeranian Medical University Ethics Committee approved the study and written informed consent was obtained from each participant.

Subjects and controls

158 male Polish power-orientated athletes of regional or national competitive standard with at least 8 years experience participating in sport were recruited for this study (48 short distance runners, 54 short distance swimmers, 56 weightlifters).

For controls, samples were prepared from 254 unrelated volunteers (male students from the University of Szczecin aged 19-23 yrs). The athletes and controls were all Caucasian to ensure no likely racial gene skew and to overcome any potential problems of population stratification.

Genotyping

Genomic DNA was extracted from oral epithelial cells using GenElute Mammalian Genomic DNA Miniprep Kit (Sigma, Germany) according to the manufacturer's instructions.

Genotyping of the *HIF1A* Pro582Ser (rs11549465) polymorphism was performed using polymerase chain reaction (PCR). The resulting PCR products were genotyped by restriction fragment length polymorphism (RFLP).

A 197 base pair (bp) fragment of HIF1A Pro582Ser (C/T) was amplified by PCR using forward primer 5'-GACTTTGAGTTTCA CTTGTTT-3' and reverse primer 5'-ACTTGCGCTTTCAGGGCTTGCGGAA CTGCTT-3' [1]. The PCR mixture (total volume 20 μl) contained 0.2 mmol/L of each primer (Genomed, Poland), 200 mM each dNTP (Novazym, Poland), 0.5 U Taq polymerase (Novazym, Poland), and 100 ng of genomic DNA. The temperature-time profile was the same as described by Eynon et al. [6]. The amplified fragment subsequently underwent digestion by NmuCI enzyme (Fermentas, Lithuania) in conditions recommended by the supplier. The digested products were visualized by 3% agarose gel electrophoresis.

Statistical analysis

Genotype distribution and allele frequencies between the groups of athletes and controls were compared and significance was assessed by χ^2 test using STATISTICA 9 statistical software. *P* values of < 0.05 were considered statistically significant.

RESULTS

HIF1A genotype distribution among athletes and controls was in Hardy-Weinberg equilibrium, making selection bias less likely. Genotype distribution results of the control group (*Pro/Pro* – 81.89%; *Pro/Ser* – 18.11%; *Ser/Ser* – 0.00%) were similar to those reported in previous studies on Caucasian populations [1,5]. The distributions of the HIF1A genotypes and alleles are given in Table 1.

The genotype distribution among the whole cohort of athletes (65.82% Pro/Pro, 34.18% Pro/Ser, 0.00% Ser/Ser) was significantly different to that among sedentary controls (81.89% Pro/Pro, 18.11% Pro/Ser, 0.00% Ser/Ser; P = 0.0002).

When only weightlifters were considered, the *P* value for genotype distribution (67.86% Pro/Pro, 32.14% Pro/Ser, 0.00% Ser/Ser) was 0.01. This trend was even stronger ($P = 0.0002$) when comparing the short distance swimmers with the control group. The genotype distribution differences were not statistically significant only when short distance runners were compared with controls ($P = 0.08$).

TABLE 1. FREQUENCY OF HIF1A ALLELES AND GENOTYPES

Group	n	Genotypes, %			
		Pro/Pro	Pro/Ser	Ser/Ser	Ser allele, %
Short distance runners	48	70.83	29.17	0.00	14.58
Short distance swimmers	54	59.26	40.74**	0.00	20.37*
Weightlifters	56	67.86	32.14*	0.00	16.07
All athletes	158	65.82	34.18**	0.00	17.09*
Control	254	81.89	18.11	0.00	9.05

Note: *p<0.05, **p<0.001 compared to control

The genotype distributions among the subgroups of athletes were not significantly different (short distance runners vs. short distance swimmers, $P = 0.18$; short distance runners vs. weightlifters, $P = 0.74$; short distance swimmers vs. weightlifters, $P = 0.29$).

A significant excess of the Ser582 allele was noted in the whole cohort of athletes (17.09%, $P = 0.03$). This trend was similar when comparing the controls (9.05%) with the allele frequency in the short distance swimmers (20.37%, $P = 0.03$).

The incidence of the *HIF1A* Ser582 allele in short distance runners (14.58% vs. 9.05%; $P = 0.39$) and weightlifters (16.07 vs. 9.05%; $P = 0.20$) was not significantly higher than in the control group.

The allele frequency among the subgroups of athletes was not significantly different (short distance runners vs. short distance swimmers, $P = 0.23$; short distance runners vs. weightlifters, $P = 0.76$; short distance swimmers vs. weightlifters, $P = 0.35$).

DISCUSSION

Reports regarding the interaction between the *HIF1A* Pro582Ser polymorphism and power-orientated sports are limited. The majority of articles concerning the role of the *HIF1A* gene for sport performance indicate this gene as a genetic marker associated with endurance athlete status [5,8,11]. To date only [1,6] have investigated the hypothesis that *HIF1A* Pro582Ser genotype distribution may differ for power-orientated athletes and controls. The present study is the first report regarding the *HIF1A* gene Pro582Ser polymorphism in Polish athletes.

In the present study, we investigated the association between the *HIF1A* Pro582Ser polymorphism and athletic performance. The *HIF1A* gene was taken into consideration as a genetic marker of athletic ability because of its proposed role in promoting a shift of type I (oxidative) muscle fibres to type IIX (glycolytic) muscle fibres [1], increasing hypoxic resistance of cells [13], but also its role as a transcription factor regulating gene expression [4,19].

Our data on *HIF1A* Pro582Ser polymorphism association with power-orientated athletes are in line with findings which demonstrated a relationship between HIF1A Ser582 allele and high activity of *HIF1A* transcription factor and high level of glycolysis [3,13], which is considered as the central source of anaerobic energy in power performance. The *HIF1A* Ser582 allele in this case can be regarded as an allele favouring the development and manifestation of speed and power capacities [1].

Ahmetov et al. [1] found a correlation between the HIF1A Pro582Ser polymorphism and status of Russian sprint/strength athletes. The frequency of the HIF1A Ser582 allele was significantly higher in weightlifters than in 920 controls (17.9 vs. 8.5%; $P=0.001$) and increased with their levels of achievement from 14.7% in sub-elite athletes to 25.0% in high elite athletes. Our findings also showed the significantly higher frequency of *HIF1A* Ser582 in power-orientated athletes than in controls (17.09 vs. 9.05%; $P = 0.03$). We also observed the same range of percentage of *HIF1A* Pro/Ser genotype frequency in Polish power-orientated athletes compared to Russian athletes reported by Ahmetov et al. [1] (34.1% vs. 32.4%, $P = 0.80$).

On the other hand, our findings were in contrast to those reported by Eynon *et al.* [6], who did not find any statistically significant differences in *HIF1A* genotype distribution and Ser582 allele frequency between groups of endurance athletes, sprinters, and controls. The percentage comparison of *HIF1A* Ser582 allele between power-orientated athletes and controls was 14.8% *vs.* 17.4%, $P = 0.59$. Additionally, no statistical differences were found between the subgroups of top-level endurance athletes and national-level endurance athletes, or between top-level and national-level sprinters. In this case, a possible explanation for the differences in *HIF1A* genotype distribution and Ser582 allele frequency observed in Polish and Russian power-orientated athletes compared to sportsmen reported by Eynon et al. [6] may be the fact that their results were unique to the selected population or the Israeli ethnic group.

It is worth noting that differences in the genotype distribution and allele frequency between the subgroups were not statistically significant. This fact may indirectly suggest that the *HIF1A* Pro582Ser polymorphism may also have the same level of importance in all athlete subgroups.

Our study was not without limitations. The investigated groups were not large, owing to limitations imposed by the small number of power-orientated athletes who agreed to participate in the research and available in Poland. Unfortunately, the relatively small size of subgroups in this study may cause ambiguity of the obtained results (relatively low statistical test power).

Additionally it is worth noting that elite athletic performance is a polygenic trait, with over 20 polymorphisms suggested to influence the result of endurance [7]. The genetic marker analysed independently is likely to make a limited contribution to an 'elite' phenotype: it seems more likely that such status depends on the simultaneous presence of multiple such variants [2].

The investigated group consisted mostly of regional or national competitive standard athletes, although with at least 8 years experience participating in sport. On the other hand, even in this group our study indicates that the HIF1A Ser582 allele could be one of the factors influencing the power exercise performance. It seems possible that the indicated trend may be even stronger in elite and high elite athletes. Therefore, these results should be supported with more experimental studies on *HIF1A* polymorphisms in elite athletes. Furthermore, genetic association studies must always be interpreted with caution.

CONCLUSIONS

In conclusion, our results indicate a higher frequency of the *HIF1A* Ser582 allele and Pro/Ser genotype in the group of power-orientated athletes than the control group. These data may suggest that the Ser allele is associated with power athlete status, and therefore it may be taken into consideration for inclusion in the group of performance enhancing polymorphisms as a factor beneficial to power performance.

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