The *HIF1A* gene Pro582Ser polymorphism in Russian strength athletes

Running head: **The *HIF1A* gene variant in Russian athletes**

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Abstract

Hypoxia-inducible factor 1-α (HIF-1α; encoded by *HIF1A* gene) controls a number of genes that are implicated in various cellular functions including glycolysis, cell proliferation and differentiation. The rs11549465 C>T polymorphism in the *HIF1A* gene which produces the amino acid substitution Pro582Ser increases protein stability and transcriptional activity, and therefore, improves glucose metabolism. The aim of our study was to investigate the association between the *HIF1A* Pro582Ser polymorphism and elite strength athlete status. A total of 208 Russian strength athletes (122 weightlifters and 86 wrestlers) of regional or national competitive standard and 1,413 controls were genotyped using PCR-RFLP method. We found that the frequency of the *HIF1A* 582Ser variant was significantly higher in weightlifters (13.1%, P = 0.0031), as well as in wrestlers (15.7%, P = 0.0002) compared to controls (7.5%). Additionally, the highest (21.1%; P = 0.0052) frequency of the 582Ser variant was found in a group of elite strength athletes. Thus, our study provides evidence for an association between the *HIF1A* gene Pro582Ser polymorphism and elite strength athlete status. Although more replication studies are needed, the preliminary data suggest an opportunity to use the analysis of *HIF1A* polymorphism along with other gene variations and standard phenotypic assessment in sports selection.

Key words: handgrip strength, wrestling, weightlifting, anaerobic glycolysis

INTRODUCTION

Glycolysis is the central source of anaerobic energy in humans, and this metabolic pathway is regulated under low-oxygen conditions by the nuclear transcription factor hypoxia-inducible factor 1α (HIF-1α; encoded by *HIF1A* gene). HIF-1α controls the expression of over 100 genes implicated in various cellular functions including glucose metabolism, apoptosis, cell proliferation and differentiation, matrix metabolism, vascular tone, erythropoiesis/iron metabolism and angiogenesis (reviewed in Ke and Costa, 2006).

Although expression of HIF-1α is mainly controlled by oxygen tension, it is also regulated by oxygen-independent mechanisms. For example, HIF-1α has been shown to be activated in response to insulin-like growth factor-1 (IGF-1) (Punglia et al., 1997; Fukuda et al., 2002; Slomiany and Rosenzweig, 2006). In terms of adaptation to strength training it is also important to note that HIF1α regulates expression of genes coding for some of the growth factors such as insulin-like growth factor-2 (IGF-2) and transforming growth factor-α (TGF-α) (Feldser et al., 1999; Krishnamachary et al., 2003).

Expression of HIF1α mRNA and protein is greater in more glycolytic compared with more oxidative muscles in animal models (Pisani and Dechesne, 2005). A lower proportion of type IIA fibres in the soleus muscles of HIF-1α knockout mice was detected as well as a metabolic shift away from glycolysis toward oxidation, and as a consequence, improved endurance capacity (Mason et al. 2004). Conversely, Lunde et al. (2011) have shown that when HIF-1α was overexpressed for 14 days after somatic gene transfer in adult rats, a slow-to-fast transformation in phenotype was observed.

In humans, a missense polymorphism in the *HIF1A* gene, Pro582Ser, is present in exon 12 (rs11549465 C/T). 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 HIF1α protein stability and transcriptional activity (Tanimoto et al. 2003), and therefore, may improve glucose metabolism

and lower the risk of type 2 diabetes (Nagy et al. 2009). The *HIF1A* 582Ser variant was shown to be significantly associated with an increased proportion of fast-twitch muscle fibres in *m. vastus lateralis* of Russian all-round speed skaters (Ahmetov et al. 2008).

Given the role of HIF1α in the regulation of expression of genes implicated in glycolysis, muscle growth and muscle fibre composition, one might suggest that a functional *HIF1A* Pro582Ser polymorphism is associated with human physical performance. Indeed, Cięszczyk et al. (2011) have demonstrated that the percentage of Polish weightlifters with the 582Ser allele (Pro/Ser heterozygotes) was significantly higher than in the control group. If such an association between Pro582Ser variant and performance capability is mediated via differences in muscle fibre type proportion, the Pro allele may be advantageous for endurance athletes. Accordingly, Döring et al. (2010) reported that the Pro582 variant was associated with endurance athlete status in 316 Caucasian male elite endurance athletes in comparison with controls. These results suggest that the *HIF1A* Pro582Ser polymorphism might belong to a growing group of performance-associated polymorphisms at the elite level and could be used in genetic-based athletic talent identification.

The aim of our study was to investigate the association between the *HIF1A* Pro582Ser polymorphism and elite strength athlete status.

METHODS

Study participants

In total, 208 Russian athletes (169 male and 39 female, age 26.6 ± 0.7 yr) of regional or national competitive standard were recruited from the following sports: weightlifting (n = 122) and wrestling (n = 86). There were 19 athletes classified as ‘elite’ (ranked in the top 10 internationally) and 73 athletes were classified as ‘sub-elite’ (participants in international

competitions). The others (n = 116) were classified as 'non-elite' athletes, being regional competitors with no less than four years experience participating in their sports.

Controls were 1,413 healthy unrelated citizens of St Petersburg, Moscow, Kazan, Naberezhniye Chelny and Surgut (574 males and 839 females; 22.5 ± 0.3 yr) without any competitive sport experience. Geographic ancestry of the athlete and control groups was self-reported as 100% Caucasian. The study was approved by ethics committees of the St Petersburg State University, Kazan State Medical University and The Russian Federal Agency for Physical Culture and Sports.Additionally, written informed consent was obtained from each participant.

Genotyping

Molecular genetic analysis was performed with DNA samples obtained from epithelial mouth cells by alkaline extraction or using a DNK-sorb-A sorbent kit according to the manufacturer's instruction (Central Research Institute of Epidemiology, Moscow, Russia), depending on the method of sample collection (buccal swab or scrape). Genotyping of the *HIF1A* gene polymorphism was performed by PCR on multicanal amplificator Tercyk (DNA Technology, Moscow, Russia), according to the previously described method (Ahmetov et al. 2008). PCR primers were forward GACTTTGAGTTTCACTTGTTT and reverse ACTTGCGCTTTCAGGGCTTGCGGAACTGCTT (Litech, Russia), generating a fragment of 197 bp. PCR products were digested with NmuCI (Fermentas, Lithuania) for 12 hours at 37°C and were separated by 8% polyacrylamide gel electrophoresis, stained with ethidium bromide, and visualized in UV light. All genotyping analyses were conducted blind to subject identity.

Statistical analysis

Case-control studies remain the most common study design in sports genomics and generally involve determining whether one allele of a DNA sequence is more common in a group of elite

athletes than it is in the general population, thus implying that the allele boosts performance. Genotype distribution and allele frequencies between athletes and controls were compared using χ^2 tests. *P* values < 0.05 were considered statistically significant. Bonferroni's correction for multiple testing was performed by dividing the *P* value (0.05) with the number of tests where appropriate. Statistical analyses were conducted using GrathPad Instat software.

RESULTS

HIF1A genotype distributions in the control group (Pro/Pro – 85.4%, Pro/Ser – 14.1%, Ser/Ser – 0.5%) and amongst all athletes (Pro/Pro – 72.1%, Pro/Ser – 27.4%, Ser/Ser – 0.5%) were in Hardy-Weinberg equilibrium (controls: $\chi^2 = 0.091$; $P = 0.955$; athletes: $\chi^2 = 2.16$; $P = 0.339$). There were no significant differences in *HIF1A* genotype and allele frequencies between males and females amongst athletes and controls, nor between controls from different towns (data not shown).

Genotype distribution in the whole cohort of strength athletes showed significant differences ($P < 0.0001$) when compared to controls. The frequency of the *HIF1A* 582Ser variant (14.2% vs. 7.5%; $P < 0.0001$) was significantly higher in athletes compared to controls (Table 1). Compared with Pro/Pro carriers, the odds ratio (OR) of being a strength athlete in 582Ser variant carriers (Pro/Ser+Ser/Ser) was 2.27 (95% confidence interval (CI): 1.62-3.17, $P < 0.0001$). When considering individual sporting disciplines, the frequency of *HIF1A* 582Ser variant was significantly higher in weightlifters (13.1%, $P = 0.0031$), as well as in wrestlers (15.7%, $P = 0.0002$) compared to controls (Table 1).

Within the whole group of strength athletes, *HIF1A* 582Ser variantfrequency was significantly associated with competitive level of the athlete. The highest (21.1%; $P = 0.0052$ in comparison with controls) frequency of the 582Ser variantwas found in a group of elite strength athletes (Fig. 1).

DISCUSSION

The heritability of muscle strength has been shown to range from approximately 30 up to 80% (Thomis et al. 1998; Silventoinen et al. 2008). Muscle strength/power phenotypes are accepted to be polygenic in nature – that is, multiple genetic factors influence the observed phenotype (reviewed in Hughes et al. 2011). It has long been established that skeletal muscle hypertrophy, predominance of fast-twitch muscle fibres and high glycolytic capacity are major contributing factors to the performance of strength athletes (Tesch, 1988; Longhurst and Stebbins, 1992; Fry et al. 2003). Data suggest that the nuclear transcription factor HIF-1α may be involved in the determination of the aforementioned factors. Indeed, studies with HIF-1α knockout or overexpression rodent models have illustrated a transformation of muscle fibre composition accompanied by a shift in metabolic profile (Mason et al. 2004; Lunde et al. 2011). In addition, hypoxia induced by venous occlusion in rats causes skeletal muscle hypertrophy with fibre-type transition towards faster types and changes in contents of muscle metabolites (Kawada and Ishii, 2008). This effect may be mediated through the regulation of erythropoietin (*EPO*), vascular endothelial growth factor receptor 2 (*VEGFR2*) and/or other genes by HIF-1α. For example, Cayla et al. (2008) have demonstrated that the treatment of rats with erythropoietin induced a shift of muscle phenotype from fast glycolytic to slow oxidative. Furthermore, we have previously reported that variation in the *VEGFR2* gene is associated with muscle fibre composition in physically active men and this finding was also reflected in genotype frequencies of athletes (Ahmetov et al. 2009a). A hypertrophic effect of HIF-1α on skeletal muscle may be explained by action at the IGF1–HIF-1α–IGF2/TGFα axis (Punglia et al., 1997; Feldser et al., 1999; Fukuda et al., 2002; Krishnamachary et al., 2003; Slomiany and Rosenzweig, 2006; Gariboldi et al. 2010).

Our data suggest that the *HIF1A* Pro582Ser amino acid substitution (T allele) (with increased protein stability and transcriptional activity) is positively associated with elite strength athlete status in Russians. The results presented here are in agreement with previously reported case-control study of Polish athletes which provide evidence that *HIF1A* Pro/Ser genotype is over-represented (32.1 vs. 18.1%) in weightlifters in comparison with controls (Cięszczyk et al. 2011). Our findings are also supported by the observations that the *HIF1A* 582Ser variant was associated with an increased proportion of fast-twitch muscle fibres (Pro/Ser – 46.2 (13.8)%, Pro/Pro – 31.4 (8.2)%) in *m. vastus lateralis* of 21 Russian all-round speed skaters (14 males and 7 females; age 20.5 ± 0.5 years) (Ahmetov et al. 2008), and with higher glycolytic capacity of skeletal muscle following training (determined via measurement of several enzymes in *m. vastus lateralis* in young women) (McPhee et al. 2011). However,Eynon et al. (2010) did not find significant differences in genotype and allele frequencies of the *HIF1A* gene Pro582Ser polymorphism in 81 Israeli sprinters compared to 240 healthy controls.

There are also three studies reporting genotype distributions and allele frequencies of the *HIF1A* gene Pro582Ser polymorphism in endurance athletes. Döring et al. 2010 have shown that the frequency of Pro/Pro genotype was significantly higher in 316 Caucasian male elite endurance athletes compared to sedentary controls (83.8% vs. 74.6%; $P = 0.006$). However, in studies of 265 Russian and 74 Israeli endurance-oriented athletes no association was found (Ahmetov et al. 2009b; Eynon et al. 2010).

In summary, our study provides evidence for the association between the *HIF1A* gene Pro582Ser polymorphism and elite strength athlete status. Further studies are needed to replicate our findings in strength and power athletes as well as to investigate the association of the *HIF1A* Pro582Ser polymorphism with strength and skeletal mass related phenotypes.

PRACTICAL APPLICATIONS

We have shown that the odds ratio of being a strength athlete in *HIF1A* 582Ser allele carriers was 2.27. Although more replication studies are needed, the preliminary data suggest an opportunity to use the analysis of *HIF1A* polymorphism along with other gene variations and standard phenotypic assessment in sports selection.

REFERENCES

1. Ahmetov, II, Hakimullina, AM, Lyubaeva, EV, Vinogradova, OL, and Rogozkin, VA. Effect of HIF1A gene polymorphism on human muscle performance. *Bull Exp Biol Med* 146: 351–353, 2008.
2. Ahmetov, II, Hakimullina, AM, Popov, DV, Lyubaeva, EV, Missina, SS, Vinogradova, OL, Williams, AG, and Rogozkin, VA. Association of the VEGFR2 gene His472Gln polymorphism with endurance-related phenotypes. *Eur J Appl Physiol* 107(1): 95-103, 2009a.
3. Ahmetov, II, Williams, AG, Popov, DV, Lyubaeva, EV, Hakimullina, AM, Fedotovskaya, ON, Mozhayskaya, IA, Vinogradova, OL, Astratenkova, IV, Montgomery, HE, and Rogozkin, VA. The combined impact of metabolic gene polymorphisms on elite endurance athlete status and related phenotypes. *Hum Genet* 126: 751–761, 2009b.
4. Cayla, JL, Maire, P, Duvallet, A, and Wahrmann, JP. Erythropoietin induces a shift of muscle phenotype from fast glycolytic to slow oxidative. *Int J Sports Med* 29(6): 460-465, 2008.
5. Cięszczyk, P, Eider, J, Arczewska, A, Ostanek, M, Leońska-Duniec, A, Sawczyn, S, Ficek, K, Jascaniene, N, Kotarska, K, and Sygit, K. The HIF1A gene Pro582Ser polymorphism in Polish power-orientated athletes. *Biol Sport* 28(2): 111-114, 2011.

6. Döring, F, Onur, S, Fischer, A, Boulay, MR, Pérusse, L, Rankinen, T, Rauramaa, R, Wolfarth, B, and Bouchard, C. A common haplotype and the Pro582Ser polymorphism of the hypoxia-inducible factor-1alpha (HIF1A) gene in elite endurance athletes. *J Appl Physiol* 108(6): 1497-1500, 2010.
7. Eynon, N, Alves, AJ, Meckel, Y, Yamin, C, Ayalon, M, Sagiv, M, and Sagiv, M. Is the interaction between *HIF1A* P582S and *ACTN3* R577X determinant for power/sprint performance? *Metabolism* 59(6): 861-865, 2010.
8. Feldser, D, Agani, F, Iyer, NV, Pak, B, Ferreira, G, and Semenza, GL. Reciprocal positive regulation of hypoxia-inducible factor 1alpha and insulin-like growth factor 2. *Cancer Res* 59: 3915-3918, 1999.
9. Fry, AC, Schilling, BK, Staron, RS, Hagerman, FC, Hikida, RS, and Thrush, JT. Muscle fiber characteristics and performance correlates of male Olympic-style weightlifters. *J Strength Cond Res* 17(4): 746-754, 2003.
10. Fukuda, R, Hirota, K, Fan, F, Jung, YD, Ellis, LM, and Semenza, GL. Insulin-like growth factor 1 induces hypoxia-inducible factor 1-mediated vascular endothelial growth factor expression, which is dependent on MAP kinase and phosphatidylinositol 3-kinase signaling in colon cancer cells. *J Biol Chem* 277: 38205–38211, 2002.
11. Gariboldi, MB, Ravizza, R, and Monti, E. The IGFR1 inhibitor NVP-AEW541 disrupts a pro-survival and pro-angiogenic IGF-STAT3-HIF1 pathway in human glioblastoma cells. Biochem Pharmacol 80(4): 455-462, 2010.
12. Hughes, DC, Day, SH, Ahmetov, II, and Williams, AG. Genetics of muscle strength and power: polygenic profile similarity limits skeletal muscle performance. *J Sports Sci* 29(13): 1425-1434, 2011.

13. Kawada, S, and Ishii N. Changes in skeletal muscle size, fibre-type composition and capillary supply after chronic venous occlusion in rats. Acta Physiol 192(4): 541-549, 2008.
14. Ke, Q, and Costa, M. Hypoxia-inducible factor-1 (HIF-1). *Mol Pharmacol* 70(5): 1469-1480, 2006.
15. Krishnamachary, B, Berg-Dixon, S, Kelly, B, Agani, F, Feldser, D, Ferreira, G, Iyer, N, LaRusch, J, Pak, B, Taghavi, P, and Semenza, GL. Regulation of colon carcinoma cell invasion by hypoxia-inducible factor 1. *Cancer Res* 63: 1138-1143, 2003.
16. Longhurst JC, and Stebbins CL. The isometric athlete. *Cardiol Clin* 10(2): 281-294, 1992.
17. Lunde, IG, Anton, SL, Bruusgaard, JC, Rana, ZA, Ellefsen, S, and Gundersen, K. Hypoxia inducible factor 1 links fast-patterned muscle activity and fast muscle phenotype in rats. J Physiol 589(Pt 6): 1443-1454, 2011.
18. Mason, SD, Howlett, RA, Kim, MJ, Olfert, IM, Hogan, MC, McNulty, W, Hickey, RP, Wagner, PD, Kahn, CR, Giordano, FJ, and Johnson, RS. Loss of skeletal muscle HIF-1α results in altered exercise endurance. PLoS Biol 2: e288, 2004.
19. McPhee, JS, Perez-Schindler, J, Degens, H, Tomlinson, D, Hennis, P, Baar, K, and Williams, AG. *HIF1A* P582S gene association with endurance training responses in young women. *Eur J Appl Physiol* 111(9): 2339-2347, 2011.
20. Nagy, G, Kovacs-Nagy, R, Kereszturi, E, Somogyi, A, Szekely, A, Nemeth, N, Hosszufalusi, N, Panczel, P, Ronai, Z, and Sasvari-Szekely, M. Association of hypoxia inducible factor-1 alpha gene polymorphism with both type 1 and type 2 diabetes in a Caucasian (Hungarian) sample. BMC Med Genet 10: 79, 2009.
21. Pisani, DF, and Dechesne, CA. Skeletal muscle HIF-1alpha expression is dependent on muscle fiber type. *J Gen Physiol* 126: 173-178, 2005.

22. Punglia, RS, Lu, M, Hsu, J, Kuroki, M, Tolentino, MJ, Keough, K, Levy, AP, Levy, NS, Goldberg, MA, D'Amato, RJ, and Adamis, AP. Regulation of vascular endothelial growth factor expression by insulin-like growth factor 1. *Diabetes* 46: 1619–1626, 1997.
23. Silventoinen, K, Magnusson, PK, Tynelius, P, Kaprio, J, and Rasmussen, F. Heritability of body size and muscle strength in young adulthood: a study of one million Swedish men. *Genet Epidemiol* 32(4): 341-349, 2008.
24. Slomiany, MG, and Rosenzweig, SA. Hypoxia-inducible factor-1-dependent and -independent regulation of insulin-like growth factor-1-stimulated vascular endothelial growth factor secretion. *J Pharmacol Exp Ther* 318: 666–675, 2006.
25. Tanimoto, K, Yoshiga, K, Eguchi, H, Kaneyasu, M, Ukon, K, Kumazaki, T, Oue, N, Yasui, W, Imai, K, Nakachi, K, Poellinger, L, and Nishiyama, M. Hypoxia-inducible factor-1alpha polymorphisms associated with enhanced transactivation capacity, implying clinical significance. Carcinogen 24: 1779-1783, 2003.
26. Tesch, PA. Skeletal muscle adaptations consequent to long-term heavy resistance exercise. *Med Sci Sports Exerc* 20(5 Suppl): S132-134, 1988.
27. Thomis, MA, Beunen, GP, Van Leemputte, M, Maes, HH, Blimkie, CJ, Claessens, AL, Marchal, G, Willems, E, and Vlietinck, RF. Inheritance of static and dynamic arm strength and some of its determinants. *Acta Physiol Scand* 163(1): 59-71, 1998.

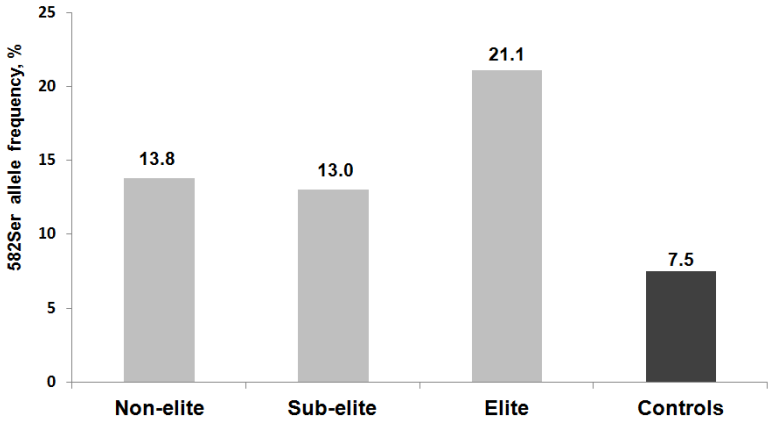

Figure 1. *HIF1A 582*Ser allele frequency amongst strength athletes stratified by competitive standard. *HIF1A 582*Ser allele frequency in controls was 7.5%. By comparison, it was 13.8% (different to controls, $P = 0.0012$), 13.0% ($P = 0.025$) and 21.1% ($P = 0.0052$) for non-elite, sub-elite and elite strength athletes, respectively.

Table 1. *HIF1A* genotype distribution and frequencies of *HIF1A* 582Ser allele in athletes and controls.

Group	*n*	*HIF1A* genotypes			*P* value	582Ser allele, %	*P* value
		Pro/Pro	Pro/Ser	Ser/Ser			
Weightlifters	122	90	32	0	0.0012*	13.1	0.0031*
Wrestlers	86	60	25	1	0.0005*	15.7	0.0002*
All strength athletes	208	150	57	1	<0.0001*	14.2	<0.0001*
Controls	1413	1207	199	7	1.000	7.5	1.000

*$P \leq 0.017$, statistically significant differences (after Bonferroni's correction for multiple testing)