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Is the interaction between *HIF1A* P582S and *ACTN3* R577X determinant for power/sprint performance?

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Abstract

Hypoxia-inducible factor 1 (HIF-1) is a transcription factor that regulates gene expression in response to hypoxia and has been associated with athletic performance. The aims of this study were (1) to determine the frequency distribution of *HIF1A* Pro582Ser (rs11549465) polymorphism among 155 Israeli athletes (sprinters and endurance athletes) and 240 healthy controls and (2) to analyze the influence of the interaction between *HIF1A* Pro582Ser and *ACTN3* R577X (rs1815739) genotypes on sprint performance. There were no differences across the *HIF1A* genotype and allele frequencies among endurance athletes, sprinters, and controls. Similarly, no differences were found between the subgroups of top-level and national-level endurance athletes, or between top-level and national-level sprinters. Conversely, interaction effects were found between *HIF1A* Pro582Ser and *ACTN3* R577X polymorphisms and sprinters. The proportion of *HIF1A* Pro/Pro + *ACTN3* R/R genotypes was significantly higher in sprinters than in endurance athletes and healthy controls ($P = .002$). In addition, the odds ratio for *HIF1A* Pro/Pro + *ACTN3* R/R genotype carriers being a sprinter was 2.25 (95% confidence interval, 1.24-4.1); and that for *HIF1A* Pro/Pro + *ACTN3* R/R genotype carriers being an endurance athlete was 0.5 (95% confidence interval, 0.2-1.24). We conclude that *HIF1A* Pro582Ser polymorphism by itself is not critical in determining sprint performance. However, sprinter performance is determined by the interaction between the wild-type *HIF1A* Pro/Pro genotype and *ACTN3* RR genotype.

1. Introduction

Favorable genetic endowment together with environmental factors seems to be necessary for attaining the highest level of athletic performance. Although sprint performance is likely a polygenic trait, only a few single nucleotide polymorphisms (SNPs), namely, ACTN3 R577X [1-4], ACE I/D [5-7], and, more recently, eNOS −786 T/C [8] and IL-6 −174G/C [9], were found to be associated with sprint performance.

Hypoxia-inducible factor 1 (HIF-1) is a transcription factor that is unique among mammalian transcription factors with respect to the demonstrated specificity and sensitivity of its induction by hypoxia [10]. Hypoxia-inducible factor 1 is part of a family of heterodynamic basic helix-loop-helix proteins, which is composed of 2 subunits, HIF-1α and HIF-1β. Expression levels of the HIF-1α subunit are precisely regulated by cellular O_2 concentration such that levels of HIF-1α protein and HIF-1 DNA-binding activity increase exponentially as O_2 concentration decreases [11]. The α subunit of HIF-1 also promotes cell survival and angiogenesis, and was suggested to influence glucose metabolism [12]. In connecting these data to muscle function, HIF-1 α messenger RNA and protein levels were found to be constitutively higher in type IIX muscle fibers, which have a high fatigability compared with the more oxidative type 1 muscle fibers, which have a low fatigability [13].

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A functional SNP was identified in the *HIF1A* gene that encodes the α subunit of HIF1 protein, resulting in the replacement of proline (Pro) with serine (Ser) at amino acid 582 [14]. The rare Ser582 allele, rather than the wild-type Pro582 allele, was previously associated with increased transcription activity and stability of HIF1A protein. Ahmetov et al [15] have recently found that carriers of the Pro/Ser genotype had a significantly higher percentage of type IIX muscle fibers than those homozygous for the Pro582 allele. Therefore, the Ser582 allele may increase the hypoxic resistance of cells, which would result in high glycolytic potentialities [16].

Because sprint performance requires a large amount of fast-twitch type IIX muscle fibers [17], it can be assumed that the proportion of the Ser582 allele will be higher among sprinters compared with endurance athletes and sedentary controls. Therefore, the purposes of the present study were (1) to compare the frequency distribution of the *HIF1A* Pro582Ser (rs11549465) polymorphism between athletes of sports with different demands (endurance vs sprinters) as well as between athletes of competitive levels (elite level vs national level) and (2) to test the influence of the interaction between the *HIF1A* Pro582Ser and the *ACTN3* R577X (rs1815739) genotypes because the *ACTN3* R577X polymorphism was previously reported to influence power-oriented top-level athletic performance in a broad variety of ethnic groups [1-4].

2. Methods

2.1. Subjects

The study followed recent recommendations for replicating genotype-phenotype association studies [18]. Owing to limitations, genotyping was not performed in 2 independent laboratories using different methodology. One hundred fifty-five track and field athletes (119 men and 36 women, age = 35.9 ± 12.2 years) volunteered to participate in the study. Athletes were included in the study sample only if they had participated in national/international track and field championships. The control group consisted of 240 nonathletic healthy individuals (167 men and 73 women) who were randomly selected from the Israeli population. Controls were not engaged in physical activity on a regular basis. Athletes were divided into 2 groups: (1) an endurance-type group that included 74 long distance runners (60 men and 14 women) whose main events were the 10 000-m run and the marathon and (2) a sprint-type group that included 81 sprinters (59 men and 22 women) whose main event was the 100- to 200-m dash. According to their individual best performances, athletes within each group were further divided into 2 subgroups: elite level (those who had represented Israel in world track and field championships or in the Olympic Games; 28 men and 18 women) and national level (91 men and 18 women). All subjects, athletes and nonathletes, were Israeli whites for at least 3 generations, with an equivalent ratio of mixed Jews coming from Arab countries (non-Ashkenazi) and Jews coming from Europe (Ashkenazi) (2:1). The study was approved by the Helsinki Committee, the formal ethics committee of the Hillel-Yaffe Medical Center, Hadera, Israel, according to the Declaration of Helsinki. A written informed consent was obtained from each participant.

2.2. Genotyping

Genomic DNA was extracted from peripheral EDTA-treated anticoagulated blood using a standard protocol. Genotyping of the *HIF1A* Pro582Ser (rs11549465) polymorphism was performed using polymerase chain reaction (PCR). The resulting PCR products were genotyped (in the Genetics and Molecular Biology Laboratory of the Zinman College of Physical Education and Sport Sciences at the Wingate Institute, Netanya, Israel) by restriction fragment length polymorphism. Briefly, a 197–base pair (bp) fragment of *HIF1A* Pro582Ser (C/T) was amplified by PCR using primers F 5′-GACTTTGAGTTTCACTTGTTT-3′ and R 5′-ACTTGCGCTTTCAGGGCTTGCG-GAACTGCTT-3′. The PCR was performed by first denaturation at 94°C for 5 minutes, 34 cycles of denaturation at 94°C for 60 seconds, annealing at 55°C for 60 seconds, extension at 72°C for 1 minute, and a final extension step of 10 minutes at 72°C. The amplified fragment subsequently underwent digestion by *Tsp451* (New England Biolabs, Beverly, MA) in a condition recommended by the supplier. The digested products were then electrophoresed in a 3% agarose gel. This method yields a 197-bp fragment in the presence of the T (Ser) allele, and 154 and 43 bp in the presence of the C (Pro) allele.

The *ACTN3* R577X polymorphism was genotyped according to a previously reported method [2]. To ensure proper internal control, for each genotype analysis, we used positive and negative controls from different DNA aliquots that were previously genotyped by the same method, according to recent recommendations for replicating genotype-phenotype association studies [18]. The restriction fragment length polymorphism results were scored by 2 experienced and independent investigators who were blind to the subject data.

2.3. Data analysis

The SPSS statistical package, version 17.0, was used to perform all statistical evaluations (SPSS, Chicago, IL). Allele frequencies were determined by gene counting. A Pearson χ^2 test, Yates corrected χ^2 test, or Fischer exact test was used to confirm that the observed genotype frequencies were in Hardy-Weinberg equilibrium and to compare the *HIF1A* Pro582Ser alleles and genotype frequencies between athletes and control subjects. One of these tests was also used to examine the interaction between the *HIF1A* Pro582Ser and the *ACTN3* R577X genotypes in relation to sprint performance and in relation to the sprinter's level of

Table 1
Genotype and allele frequencies of *HIF1A* Pro582Ser polymorphism in all groups

Athlete groups	n	Genotype			Allele frequencies	
		Pro/Pro	Pro/Ser	Ser/Ser	Allele Pro	Allele Ser
Endurance	74	53 (72)	21(28)	0 (0)	127 (0.86)	21 (0.14)
Sprinters	81	59 (73)	20 (25)	2 (2)	138 (0.85)	24 (0.15)
Control	240	173 (72)	63 (26)	4 (2)	409 (0.85)	71 (0.15)

Data are absolute and relative values (within parentheses). $\chi^2 = 1.85$, $df = 2$, and $P = .76$ for overall differences in genotype frequencies. $\chi^2 = 0.04$, $df = 1$, and $P = .98$ for overall differences in allele frequency.

performance. A logistic regression analysis was set to calculate the odds ratio for the interaction of both polymorphisms in sprint athletes and in control subjects. The level of significance was set at $P < .05$.

3. Results

The complete data on allele and genotype frequencies of the *HIF1A* Pro582Ser polymorphism are shown in Table 1. The genotype subtype did not differ by sex in the athletes group ($\chi^2 = 0.7$, $df = 2$, $P = .71$) or in the control group ($\chi^2 = 1.88$, $df = 2$, $P = .39$). Because the Israeli population includes whites who are mixed non-Ashkenazi and Ashkenazi, we confirmed that there was an equivalent ratio of non-Ashkenazi and Ashkenazi descent in each group (2:1) and that there were no differences across *HIF1A* genotype between non-Ashkenazi and Ashkenazi descendants ($\chi^2 = 0.07$, $df = 2$, $P = .9$). *HIF1A* genotype distribution was in agreement with the Hardy-Weinberg equilibrium within the endurance athletes ($P = .33$), the sprinters ($P = .9$), and the control group ($P = .64$). Genotype distribution and allele frequencies were similar in the groups of endurance athletes, sprinters, and controls (Table 1). Similarly, 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 (Table 2). However, interaction effects were found between *HIF1A* Pro582Ser and *ACTN3* R577X polymorphisms and sprinters (Table 3). *HIF1A* Pro/Pro + *ACTN3* R/R genotypes were more frequently found in the sprinters than in the control group (Figs. 1 and 2).

Table 3
Combined *ACTN3* R577X and *HIF1A* Pro582Ser polymorphisms genotype frequencies within the endurance athletes, the sprinters, and the control group

ACTN3 Genotype	HIF1A genotype	Endurance athletes (n = 74)	Sprinters (n = 81)	Controls (n = 240)
RR	Pro/Pro	6 (8.1)	23 (28.4)	36 (15)
RR	Pro/Ser	8 (10.8)	9 (11.1)	13 (5.4)
RX	Pro/Pro	30 (40.5)	23 (28.4)	104 (43.3)
RX	Pro/Ser	6 (8.1)	7 (8.6)	42 (17.5)
RX	Ser/Ser	0 (0)	2 (2.5)	3 (1.3)
XX	Pro/Pro	17 (23)	13 (16)	33 (13.8)
XX	Pro/Ser	7 (9.5)	4 (4.9)	8 (3.3)
XX	Ser/Ser	0 (0)	0 (0)	1 (4)

Data is presented as absolute and relative values (within parentheses). $\chi^2 = 33.3$, $df = 14$, and $P = .003$ for overall combined genotype distribution. $\chi^2 = 12.4$, $df = 2$, and $P = .002$ for RR + Pro/Pro. $\chi^2 = 2.3$, $df = 2$, and $P = .33$ for RR + Pro/Ser. $\chi^2 = 3.4$, $df = 2$, and $P = .19$ for RX + Pro/Pro. $\chi^2 = 4.9$, $df = 2$, and $P = .08$ for RX + Pro/Ser. $\chi^2 = 2.5$, $df = 2$, and $P = .29$ for RX + Ser/Ser. $\chi^2 = 2.6$, $df = 2$, and $P = .27$ for XX + Pro/Pro. $\chi^2 = 3.5$, $df = 2$, and $P = .17$ for XX + Pro/Ser. $\chi^2 = 3.5$, $df = 2$, and $P = .17$ for XX + Pro/Ser. $\chi^2 = 8.0$, $df = 2$, and $P = .02$ for XX + Ser/Ser.

In the whole cohort of athletes, the odds ratio of *HIF1A* Pro/Pro + *ACTN3* R/R genotypes being a sprinter was 2.25 (95% confidence interval, 1.24-4.1); and that of the *HIF1A* Pro/Pro + *ACTN3* R/R genotypes being an endurance athlete was 0.5 (95% confidence interval, 0.2-1.24).

4. Discussion

In the present study, we investigated the association between *HIF1A* Pro582Ser polymorphism and elite athletic performance. The *HIF1A* gene was chosen as a genetic marker of athletic ability because of its proposed role in increasing transcription activity [14], promoting a shift of type I (oxidative) muscle fibers to type IIX (glycolytic) muscle fibers [15], and increasing hypoxic resistance of cells [16]. Our main findings were as follows: (1) genotype distribution and allele frequency within the *HIF1A* Pro582Ser polymorphism were similar in endurance athletes, sprinters, and sedentary controls; and (2) *HIF1A* Pro/Pro + *ACTN3* R/R genotypes were more frequently

Table 2
The *HIF1A* Pro582Ser genotype and allele frequencies in sprinters and endurance athletes according to their level of competition

Athlete groups	Competitive level	n	Genotype			Allele frequencies	
			Pro/Pro	Pro/Ser	Ser/Ser	Allele Pro	Allele Ser
Endurance	Top level	20	16 (80)	4 (20)	0 (0)	36 (0.9)	4 (0.1)
	National level	54	37 (69)	17 (31)	0 (0)	91(0.84)	17 (0.16)
Sprinters	Top level	26	20 (77)	6 (23)	0 (0)	46 (0.88)	6 (0.12)
	National level	55	39 (71)	14 (25)	2 (4)	92 (0.84)	18 (0.16)

Data are presented as absolute and relative values (within parentheses). $\chi^2 = 1.07$, $df = 2$, and $P = .58$ for genotype frequencies in top-level vs national-level sprinters. $\chi^2 = 0.65$, $df = 1$, and $P = .42$ for allele frequency in top-level vs national-level sprinters. $\chi^2 = 0.95$, $df = 2$, and $P = .33$ for genotype frequencies in top-level vs national-level endurance athletes. $\chi^2 = 0.79$, $df = 1$, and $P = .37$ for allele frequency in top-level vs national-level endurance athletes.

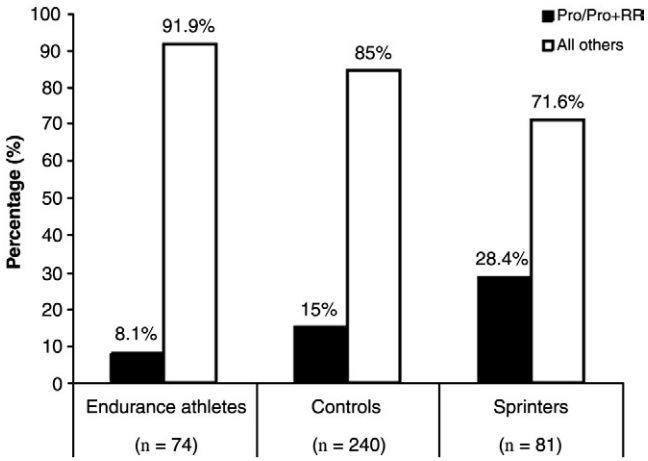

Fig. 1. Genotype frequencies of the "optimal genotype" for power/sprint phenotypes within the sprinters, the endurance athletes, and the control group. $\chi^2 = 12.5$, $df = 2$, and $P = .002$ for genotype frequencies differences in *ACTN3* RR + *HIF1A* Pro/Pro vs other genotypes between endurance athletes, sprinters, and controls.

found in the sprinters than in the endurance athletes group and the control group. These findings suggest that the *HIF1A* Pro582Ser polymorphism by itself is not a factor in determining power/sprint performance. However, the wild-type *HIF1A* Pro/Pro genotype interacts with the *ACTN3* RR genotype, which was previously associated with power/sprint performance [1-4].

Hypoxia-inducible factor 1 mediates increased glycolytic generation of adenosine triphosphate and possibly other intracellular metabolic adaptations to hypoxia [12]. Thus, one can expect that this protein will play a role in sprint events because short distance sprint and power events rely largely on the anaerobic pathways, which are especially dependent on intramuscular stored creatine phosphate, adenosine triphosphate, and glycogen [17].

Reports regarding the interaction between the *HIF1A* Pro582Ser polymorphism and the *HIF1A* transcriptional activity are limited as well as inconsistent. The Ser582 allele was associated with higher transcription activity among head and neck squamous cell carcinoma patients [16]. However, another study suggested the opposite. An in vitro reporter gene transfection experiment confirmed that those homozygous for the Ser582 allele have lower transcriptional activity than wild-type alleles at comparable expression levels [19]. Furthermore, the Ser582 allele by itself also had significantly decreased reporter gene activity at some of the concentrations, although results were less consistent than with the double mutant [19]. The cross-sectional comparison in this study revealed that a higher proportion of people showing a specific "preferred genotype" (eg, *HIF1A* Pro/Pro + *ACTN3* R/R) were more likely to be sprinters; and thus, it can be assumed that the power/sprint athletes will have higher transcription activity of the *HIF1A* gene that results in high glycolytic potentialities.

The results of the present study are not in agreement with the results of Ahmetov et al [15], who suggested that the incidence of *HIF1A* Ser582 allele was significantly higher in weight lifters than in controls. However, almost 29% of the sprinters in the present study harbored the Pro/Pro + R/R genotype as opposed to only 8% and 15% in the endurance athlete group and the control group, respectively. This emphasizes the important role of the functional *ACTN3* R/X polymorphism in power/sprint performance because it is well established that actinin-3 in these athletes is presented in higher amounts in the RR genotype than in other genotypes [4] and may be necessary for developing forceful contractions at high velocity [20].

The results of the present study also emphasize the assumption that many other yet-to-be-identified polymorphisms, which may not influence sports performance individually per se, could play an important role when combined with other variants. Furthermore, beyond genotype-phenotype associations, the effect of short, noncoding RNA molecules, namely, microRNAs, on human muscle phenotypes remains to be determined. It is now believed that microRNAs regulate skeletal muscle posttranscriptional gene expression and thus modulate important aspects of muscle function, including muscle contractility [21].

Our study was not without limitations. The group of elite athletes was relatively small owing to the small number of available athletes. Nevertheless, it consisted of highly selected endurance and sprint athletes having a unique phenotype. Furthermore, genetic association studies must always be interpreted with caution. As with any statistical analysis, there is a nontrivial possibility of a false-positive result [22].

In conclusion, the *HIF1A* Pro582Ser polymorphism is not associated with sprint performance. However, the *HIF1A* Pro/Pro + *ACTN3* R/R genotypes were more frequently found in the sprinters than in the endurance athletes group and the control group. Further investigations are needed to clarify the possible role of other polymorphisms and the combination of polymorphisms in determining athletic performance.

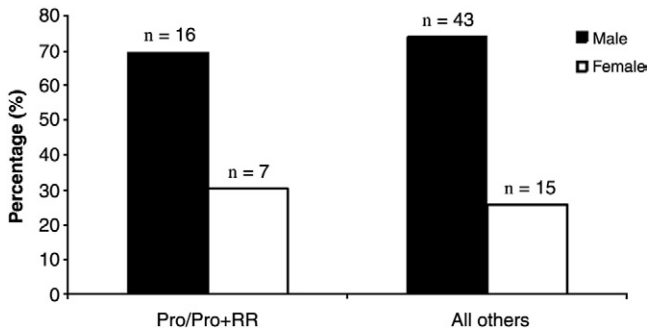

Fig. 2. Genotype frequencies of the "optimal genotype" for power/sprint phenotypes stratified by sex within the sprinters group. $\chi^2 = 0.18$, $df = 1$, and $P = .78$ for genotype frequencies differences in *ACTN3* RR + *HIF1A* Pro/Pro vs other genotypes between male and female.

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