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***PPARA* intron 1 A/C polymorphism and elite athlete status**

Running head: *PPARA* gene and athlete status

Abstract

Peroxisome proliferator-activated receptor alpha (*PPARα*) is involved in lipid and carbohydrates metabolism. The aim of this study was to test the possible association between the *PPARA* intron 1 A/C polymorphism (rs135539) and the acquirement of elite athlete status.. 155 Israeli athletes (male, n=119; female, n=36) and 240 healthy controls (male, n=170; female, n=70) participated in the study. The athletes' group consisted of endurance athletes (n=74) and sprinters (n=81). Genotyping was performed using PCR-RFLP on DNA from leucocytes. Results showed that genotype distribution and allele frequencies were similar (p= 0.65 for genotypes and p=0.48 for allele frequency) for the groups of endurance athletes (allele frequency: A/C 0.7/0.3), sprinters (allele frequency A/C 0.66/0.34), and controls (allele frequency A/C 0.71/0.29).,Further, no statistical differences were found between the subgroups of elite-level endurance athletes (those who had represented Israel in world track and field championships or in the Olympic Games) and national-level endurance athletes (p=0.44 for genotypes and p=0.96 for allele frequency), or between elite-level and national-level sprinters (p=0.57 for genotypes and p=0.40 for allele frequency). In conclusion, the present study found no differences in genotype and allelic frequency across *PPARA* intron 1 A/C polymorphism between endurance athletes, sprinters and controls. Further research is needed in other ethnic groups in order to verify these results.

Keywords: *PPARα*, genetics, sprinters, endurance athletes

Introduction

The search for genes that are involved in the acquirement of elite athlete status is an ongoing project, with a growing number of single nucleotide polymorphisms (SNPs) being suggested to influence the outcome of elite athletic challenge (Williams & Folland, 2008).

Peroxisome proliferator-activated receptor alpha (PPAR α) is a member of the steroid hormone receptor superfamily of ligand-activated transcription factors (Yoon, 2009). PPAR α protein is present at high levels in tissues such as liver, skeletal muscle, and myocardium, in which catabolism of fatty acids occurs (Braissant et al., 1996; Liang & Ward, 2006). Upon ligand activation, PPAR α binds to PPAR response elements (PPREs), which are located in the promoter region of target genes, thereby regulating transcription of target genes (Fruchart, 2001). Among these target genes are fatty acid uptake, binding, and activation genes, and mitochondrial β -oxidation genes, which are strongly involved in lipid metabolism (Yoon, 2009). Additionally, PPAR α regulates glucose and energy homeostasis (Guerre-Millo et al., 2000).

The peroxisome proliferator-activated receptor family also consists of PPAR γ and PPAR δ (Liang & Ward, 2006). All PPARs are subject to transcriptional coactivation by peroxisome proliferator-activated receptor gamma coactivator1 (PGC-1 α). The *PPARGCIA* Ser482Gly (Eynon et al., 2009a; Eynon et al., 2009b; Lucia et al., 2005) and the *PPARA* intron 7 G/C (Ahmetov et al., 2006) SNPs were shown to be associated with elite aerobic performance.

Previous studies have comprehensively evaluated SNPs within the *PPARA* gene and assessed their association with myocardial infarction (Reinhard et al., 2008) and type 2 diabetes (Andrulionyte et al., 2007). The minor C allele of the *PPARA* A/C SNP was found to be associated with MI, and suggested to be a predictor for the

development of type 2 diabetes. Interestingly, in both cases this association was significant only when it was considered together with other SNPs in the *PPARA* gene, using haplotype analysis. Since it was recognized that the *PPARA* gene plays a role in glucose and fatty acid metabolism, it might be that this particular polymorphism is one of the growing number of polymorphisms that were previously found to be associated with elite athletic performance.

Therefore, the purpose of this study was to analyze the frequency distribution of *PPARA* intron 1 A/C polymorphism (rs135539) in athletic and non-athletic Israeli populations, and to compare the frequency distribution of the above polymorphism between athletes of sports with different demands (endurance vs. sprinters) as well as between competitive levels (elite-level vs. national level).

Material and Methods

Subjects

The study followed recent recommendations for replicating genotype-phenotype association studies (Chanock et al., 2007). Owing to limitations, genotyping was not performed in two independent laboratories using different methodology. One hundred and fifty-five track and field athletes (119 men and 36 women, age=35.9±12.2 yrs) 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 non-athletic 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 two groups: 1) An endurance-type group that included 74 long-distance runners (60 men and 14 women) whose main events were the 10000m run and the marathon; and 2) A sprint-

type group that included 81 sprinters (59 men and 22 women) whose main events were the 100-200m dash and the long jump. According to their individual best performances, athletes within each group were further divided into two 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 non-athletes, were Israeli Caucasians for ≥ 3 generations, with an equivalent ratio of mixed Jews coming from Arab countries (non-Ashkenazi) and Jews coming from Europe (Ashkenazi) (2:1, respectively). 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.

Genotyping

Genomic DNA was extracted from peripheral EDTA treated anti-coagulated blood using a standard protocol. Genotyping of the *PPARA* intron 1 A/C (rs135539) was performed using polymerase chain reaction (PCR). A 210-bp fragment of the *PPAR α* A/C polymorphism was amplified using primers-F 5'-CCAGGGGGAGGAAAGAGTGAA -3' and -R 5'-GCCACA ACTAAGCAGGCAGTG -3'. PCR was performed by denaturation at 94°C for 5 min, 34 cycles of denaturation at 94°C for 1 min, annealing at 53°C for 1 min, extension at 72°C for 1 min, and a final extension step of 10 min at 72°C. The amplified fragment subsequently underwent digestion by Hinf I (New England Biolabs, Beverly, MA, USA) in a condition recommended by the supplier. The digested products were then electrophoresed in a 2% agarose gel. This method yields 148-bp and 62-bp fragments in the presence of the C allele, and 210-bp in the presence of the A allele. To ensure proper internal control, for each genotype analysis we used

positive and negative controls from different DNA aliquots that were previously genotyped with the same method according to recent recommendations for replicating genotype-phenotype association studies (Chanock et al., 2007). The RFLP results were scored by two experienced and independent investigators who were blind to the subject data.

Data analysis

The SPSS statistical package, version 17.0, was used to perform all statistical evaluations (SPSS Inc., Chicago, IL, USA). 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 *PPARA* A/C allele and genotype frequencies between athletes and control subjects as well as between athletes from different sports and different competitive levels. The level of significance was set at $P < 0.05$.

Results

The complete data on allele and genotype frequencies of the *PPARA* A/C polymorphism are shown in Figure 1 and 2. The genotype subtype did not differ by gender in the athletes group ($\chi^2 = 0.9$, d.f=2, $P=0.74$) or in the control group ($\chi^2 = 0.4$, d.f=2, $P=0.59$). Since the Israeli population includes Caucasians 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 the *PPARA* genotype between non-Ashkenazi and Ashkenazi descendants ($\chi^2 = 0.09$, d.f=2, $P=0.71$). *PPARA* genotype distribution was in agreement with the Hardy-Weinberg equilibrium within the endurance athletes ($P=0.13$), the sprinters ($P=0.16$), and the control group ($P=0.09$). Genotype distribution and allele frequencies were similar in the groups of endurance athletes,

sprinters, and controls (Figure 2). Further, 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 1).

Discussion

The results of the present study revealed that the genotype and allele frequency distributions of the *PPARA* A/C polymorphism were similar in the endurance athletes, the sprinters, and the control group. Furthermore, genotype and allele frequency distributions of the *PPARA* A/C polymorphism were also similar in the three groups in relation to their level of performance (national level vs. elite-level). The *PPARA* A/C polymorphism cannot be used to distinguish endurance athletes from either sprinters or sedentary individuals. Previously, genetic examinations of the *PPARA* locus have mainly focused on the intron 7 G/C variant, which has been shown to be associated with endurance performance in Russian (Ahmetov et al., 2006) and Israeli (Eynon et al., 2009b) elite endurance athletes. In the present study we have determined a possible association between *PPARA* A/C polymorphism and athletes' status in both endurance and power-oriented sport disciplines.

The mechanisms by which a single genetic variant influence the process of becoming an elite athlete remain unclear. Many genetic association studies suggest that an observed association is mediated through alterations in gene expression. It is now believed that an orchestrated signal transduction from contractile activity to the gene regulatory machinery occurs during and after exercise training (Yan, Li, & Akimoto, 2007). It was reasonable to assume that variation in the *PPARA* gene will be associated with endurance performance since it was found that *PPARA* mRNA expression was significantly higher in type I (oxidative) than type II muscle fibers (Ahmetov et al., 2006). It was well established that highly-trained endurance athletes

have a relatively high amount of slow-twitch muscle fibers, and sprinters, on the other hand, have an excess of fast-twitch fibers (Russell et al., 2003). Thus, part of the allelic association with performance phenotypes might be mediated through genotype-associated alterations in fiber type proportion.

This study further pursued the essential function of the *PPARA* gene in fatty acid metabolism. PPAR α together with PPAR δ play an important role in controlling fatty acid oxidation (Yoon, 2009). Activation of the PPAR α protein results in the increased expression of genes involved in lipid oxidation, lipoprotein metabolism, inhibition of vascular inflammation, and adipocyte differentiation (Fruchart et al., 1999). Since it is well established that endurance-trained athletes derive a greater energy contribution via aerobic metabolism, which relies mainly on circulating free fatty acids and glucose, and sprint-trained individuals rely more heavily on anaerobic sources such as intramuscular stored CP, ATP, and glucose (Nummela & Rusko, 1995), it is possible that the traits underlying the endurance phenotype and the sprint phenotypes may reflect evolutionary trade-offs between endurance athletes and sprint/power athletes (Van Damme et al., 2002). Thus, *PPARA* gene variations might partly explain not only the physiological differences between elite athletes and sedentary controls, but also the differences between athletes participating in different sports (e.g, marathon runners vs. sprinters).

We are aware that genetic association studies must be interpreted with caution, since there is a non-trivial possibility of a false positive result (Macarthur & North, 2005). Nevertheless, it should be noted that our study group consisted of highly-selected Israeli endurance and sprint/power athletes who have a unique phenotype that distinguishes them from sedentary subjects.

In conclusion, the present study found no difference in either genotype distribution or allelic frequency across *PPARA* intron 1 A/C polymorphism between endurance athletes, sprinters and controls. Further research is needed in other cohorts in order to verify these results.

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Tables:**Table 1.** *PPARA* intron 1 A/C polymorphism genotype and allele frequencies in athletes divided by subgroups.

Athlete groups	Competitive level	n	Genotype			Allele frequencies	
			AA	AC	CC	Allele A	Allele C
Endurance	Elite-level	20	8 (40)	12 (60)	0 (0)	28 (0.70)	12 (0.30)
	National level	54	25 (46.3)	26 (48.1)	3 (5.6)	76 (0.70)	32 (0.30)
Sprinters	Elite-level	26	9 (34.6)	14 (53.9)	3 (11.5)	32 (0.62)	20 (0.38)
	National level	55	23 (41.8)	29 (52.7)	3 (5.5)	75 (0.68)	35 (0.32)

Data are presented as absolute and relative values (within parentheses)

$\chi^2=1.64$, d.f.=2, $p=0.44$ for genotype frequencies between elite and national-level

endurance athletes

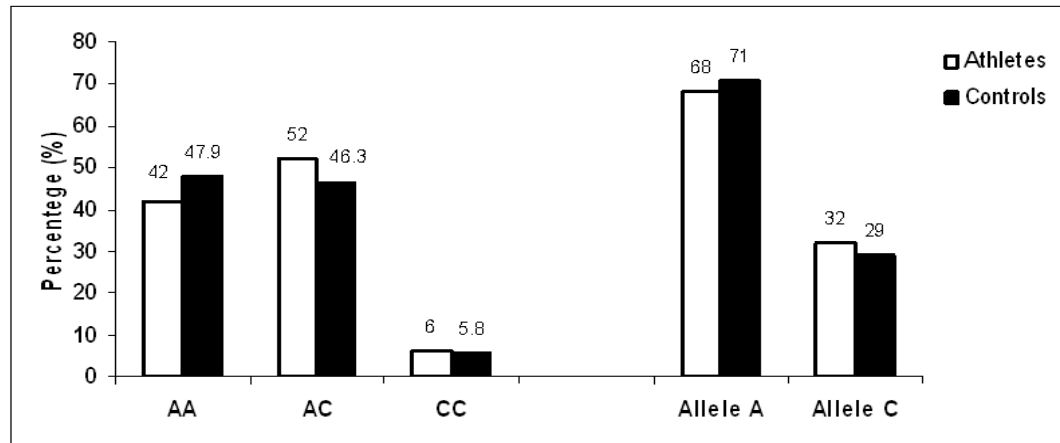
$\chi^2=0.02$, d.f.=1, $p=0.96$ for allele frequency between elite and national-level

endurance athletes

$\chi^2=1.12$, d.f.=2, $p=0.57$ for genotype frequencies between elite and national level

sprinters

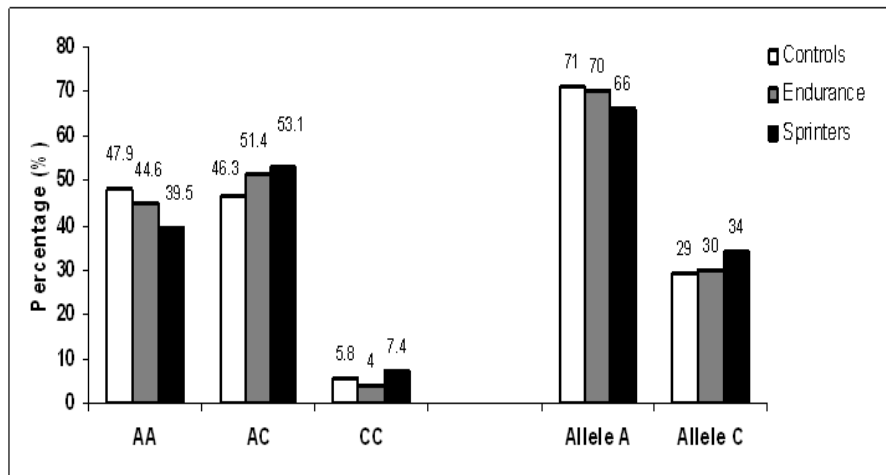
$\chi^2=0.96$, d.f.=1, $p=0.40$ for allele frequency between elite and national level sprinters

Figure legends:**Figure 1.** *PPARA* intron 1 A/C polymorphism genotype and allele frequencies in athletes and controls. Data is presented as relative values (%).

$\chi^2=1.44$, d.f.=2, $p=0.49$ for genotype frequencies between athletes and controls

$\chi^2=0.79$, d.f.=1, $p=0.37$ for allele frequency between athletes and controls

Figure 2. *PPARA* intron 1 A/C polymorphism genotype and allele frequencies in endurance athletes, sprinters, and controls. Data are presented as relative values (%).



$\chi^2=2.43$, d.f.=2, $p=0.66$ for genotype frequencies between endurance athletes, sprinters, and controls

$\chi^2=1.45$, d.f.=1, $p=0.48$ for allele frequency between endurance athletes, sprinters, and controls