The C allele in NOS3-786 T/C polymorphism is associated with elite soccer player's status

Rapid communication

Appropriate Section:

Genetics & Molecular Biology

Title: The C allele in *NOS3* -786 T/C polymorphism is associated with elite soccer player's status

Running title: *NOS3* -786 T/C polymorphism and soccer players

Abstract

The *NOS3*-786 T/C polymorphism (rs2070744) is a candidate to explain individual variations in sports related phenotypes. We determined the genotype and allele frequency of *NOS3*-786 T/C in a group of 60 male professional elite soccer players. Their results were compared with those of 100 world-class endurance athletes, 53 elite power athletes, and 100 sedentary, healthy men (controls) of the same Caucasian (Spanish) origin. There were significant differences in genotype frequencies between soccer players, controls, endurance and power elite athletes (all $P \leq 0.02$). These results were confirmed when we analysed allelic frequencies (all $P<0.01$). The likelihood of having the C allele was higher in soccer players compared with (i) controls [odds ratio (OR), 2.165, 95% confidence interval (CI): 1.362-3.441], (ii) endurance athletes (OR: 1.879, 95%CI: 1.184-2.984), and (iii) power athletes (OR: 4.032, 95%CI: 2.307-7.047). In conclusion, the -786C allele is associated with the status of being an elite soccer player, compared with non-athletic controls and also with elite endurance and power athletes. More research is needed in other groups of elite soccer players in order to replicate the results of the present study.

Key words: Genetics, athletic performance, nitric oxide synthase, polymorphism

Introduction

Top-class soccer players exhibit 'mixed' exercise phenotypes which include both endurance and power related traits. Soccer is characterized by numerous explosive short exercise bursts interspersed by brief recovery periods over an extended period of 90 minutes [13,26,27]. On the other hand, during a game, on average, a professional soccer player covers 8-12 km [3,26,27], out of which 10-20% corresponding to running bouts of near-maximal or maximal velocity performed in the determinant phases of games [3,26].

Genetic variants may explain some of the differences between athletes, in general, and non-athletes, and between athletes participating in different sport areas that involve different physiological demands [6]. Two recent studies showed a higher proportion of the R allele of the R577X polymorphism in the α-actinin-3 (*ACTN3*) gene, and of the DD genotype of the Insertion(I)/Deletion(D) polymorphism in the angiotensin-converting enzyme (*ACE*) gene, in world-class professional soccer players compared with non-athletic controls and elite endurance athletes [12,23]. In addition, Fatini et al. showed that the *ACE D* allele is associated with training-induced changes in the left ventricular mass of elite Italian soccer players [7]. However, another study found no difference in *ACE* I/D genotype frequencies between elite Korean soccer players and non-athletic controls [19]. The results of most of these studies suggest that on-field achievements of elite soccer players might be influenced not only by environmental factors, such as training, nutrition and motivation, but also by specific genetic factors.

The nitric oxide synthase 3 (*NOS3*) gene, located on human chromosome 7q35–36, encodes endothelial nitric oxide synthase (eNOS), which converts L-arginine to L-citrulline and nitric oxide (NO). NO is a well-known vasodilator that

plays a key role in blood supply to the tissues, including working muscles [10]. It has been demonstrated that an acute bout of exercise enhances the production of NO, and thereby contributes to skeletal muscle vasodilatation [4,21]. NO is involved in cardio-protection [20], myocardial respiration [15,16], and in insulin-independent glucose uptake into the working muscle fibres [17]. This molecule also modulates the kinetics of oxygen consumption during exertion [29]. Recent research demonstrated that eNOS(–/–) mice had significantly lower oxygen consumption and defective mitochondria compared with non-transgenic controls, as evidenced by decreased beta-oxidation [14]. For the abovementioned reasons, the *NOS3* gene is a candidate to be associated with sports performance. Notably, the *NOS3* -786 T/C polymorphism results in altered transcription and promoter activity [18], and is strongly involved in skeletal muscle glucose uptake during exercise [17]. Studies performed in Spanish and Italian athletes showed that the -786 T allele (which leads to higher gene transcription and promoter activity) is associated with power-oriented athletic status [8,25], whereas another study suggested the -786C allele results in reduced *NOS3* gene transcription and promoter activity and is associated with improved aerobic capacity in hypertensive men [2].

Owing to the important role of NO in muscle adaptation to exercise, as well as to the fact that the *NOS3*-786 T/C polymorphism is a candidate to explain individual variability in athletic performance, we hypothesised that the genotypes of this polymorphism are associated with the status of being an elite soccer player.

Material & Methods

Participants

The population comprised the following participants (all males) from Spain and with the same descent (Spanish Caucasians) for ≥ 3 generations:

i. 60 professional soccer players (aged 17-32 years) from the best soccer team according to FIFA (International Federation of Football Association). Eleven of them had won the Europe Champions League at least once and two had won the Soccer World Cup.

ii. 100 world-class endurance athletes aged 20-39 years (50 endurance runners and 50 professional road cyclists). All the endurance runners had participated in at least one Olympiad, and some were Olympic finalists or Europe/World Champions; the cyclists were all Tour de France finishers, including top-3 finishers.

iii. 53 elite power athletes aged 20-33 years (38 sprinters, 10 jumpers, 5 throwers). This group included 40 top national level and 13 Olympic level athletes.

iv. 100 healthy non-athletic controls aged 19-32 years. All were students from the same university (*Universidad Europea de Madrid*, Spain). Inclusion and exclusion criteria for this group were to be free of any diagnosed cardiorespiratory disease and not to be engaged in competitive sports or in formal, supervised exercise training.

Written consent was obtained from each participant. The study was approved by the ethics committee of the *Universidad Europea de Madrid*, Spain, and was performed according to ethical standards in sport and exercise science research [9].

Genotyping

Genotyping of the *NOS3* -786 T/C (rs2070744) in soccer players was performed in the Ariel University Center, Israel using the polymerase chain reaction (PCR). The primers were: F-AGTTTCCCTAGTCCCCCATGC and R-CCACACCCCCATGACTCAAGT. The amplified fragment subsequently underwent digestion by *NgoMI*. 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.

Genotyping of the *NOS3* -786 T/C polymorphism (rs2070744) in endurance athletes, power-athletes, and controls was previously performed in another laboratory (*Progenika Biopharma*, *Parque Tecnológico de Zamudio*, Derio-Vizcaya, Spain). Genotyping was performed with a newly developed low-density DNA microarray based on allele-specific probes. The design, fabrication, validation and analysis of the arrays were performed following the procedure detailed elsewhere [28] with minor modifications. The PCR products were fluorescently labelled and hybridized to the DNA microarray in an automated platform (Tecan HS4800, Mannedorf, Switzerland). Finally, the microarrays were scanned (Innopsys S.A., Carbonne, France) and we used a developed software that converts the intensity of the spots into the genotype of the polymorphism [28]. For genotyping control, sample analysis was made together with a DNA control processing with a known genotype of the *NOS3* -786 T/C polymorphism. DNA control genotypes were ensured to be correct before considering the analysis of the samples.

Statistical analysis

We used the χ^2 test to compare the genotype and allele frequency of the *NOS3* -786 T/C polymorphism in the four study groups. Between-group comparisons of genotype and allele frequencies were corrected for multiple comparisons using the Bonferroni method, in which the threshold *P*-value is obtained by dividing 0.05 by the number of comparisons. We conducted binary logistic regression analysis to determine the association between alleles and elite sports player's status. All statistical analyses were performed using the PASW (v. 18.0 for WINDOWS, Chicago).

Results

Genotype distributions were in Hardy-Weinberg equilibrium (HWE) in controls (χ^2=0.71, *P*=0.397) and soccer players χ^2=0.058, *P*=0.809) but not in the endurance (χ^2=3.88, *P*=0.048) or in the power group (χ^2=5.296, *P*=0.021). The genotype frequencies of the studied polymorphism in Spanish controls and athletes are depicted in Table 1. There were significant differences in genotype frequencies between soccer players, controls, endurance and power elite athletes (all $P \leq 0.02$, Bonferroni threshold, 0.05/3=0.02).These results were confirmed when we analysed allele frequencies (Figure 1). The likelihood of having the C allele was higher in soccer players compared with controls [odds ratio (OR), 2.165, 95% confidence interval (CI): 1.362-3.441], endurance athletes (OR: 1.879, 95%CI: 1.184-2.984), and power athletes (OR: 4.032, 95%CI: 2.307-7.047).

Discussion

In the present study we have investigated the association between variants of the *NOS3* -786 T/C polymorphism and elite soccer player's status. Our main finding was the overrepresentation of the -786CC genotype and of the -786C allele in Spanish elite soccer players compared to ethnically-matched elite endurance athletes, elite power athletes, and controls.

It is not easy to find a biological explanation for the association we found between the C allele of the *NOS3* -786 C/T polymorphism and elite soccer player's status, and especially with the fact that this allele was overrepresented in soccer players compared with the endurance athletes' group. Studies performed in Spanish and Italian athletes showed that the -786 T allele (which leads to increased gene function) is associated with power-oriented elite athletic status [8,25]. These findings

might partly explain the role that NO and NOS play in muscle hypertrophy, which is clearly an important phenotype trait in power athletic events [8]. For instance, NOS activity is important for the up-regulation of contractile protein gene expression [24]. However, studies are more controversial with regards to the possible role of the -786 C allele (which leads to reduced gene expression and promoter activity) on sports performance, and on endurance-related phenotype traits, notably vascular resistance or oxidative metabolism.

Though the-786C allele has been associated with disease phenotype traits which indicated on reduced blood flow in the cardiovascular system, i.e. hypertension [11] and coronary spasm [18], it could also have beneficial effects in response to aerobic exercise in healthy people. Data et al. reported that endurance exercise training lowered forearm vascular resistance and increased forearm blood flow in healthy, young, sedentary women who were carriers of the -786C allele [5]. More recently, Augeri et al. [2] found that the -786C allele is associated with blood pressure reductions immediately following low to moderate intensity aerobic exercise. On the other hand, eNOS(–/–) mice had significantly lower oxygen consumption compared with their wild-type counterparts [14]. Thus, more research, using mechanistic approaches (e.g. analysis of gene expression in response to endurance exercise) is needed to determine the potential association (whether positive or not) of the -786 C allele with endurance-related phenotype traits in elite athletes.

In the present study over 60% of the elite soccer players harboured the -786C allele, compared to a significantly lower proportion (30%) in the power-oriented athletes. It remains to be identified which exercise phenotype traits (whether related to aerobic or anaerobic pathways) are favoured by the C allele. Unlike "individual" sport specialties (i.e. most ‘pure’ endurance and power events), which at the elite

competition level require that the athletes exhibit unique levels of performance in either endurance or power related phenotype traits, top-class soccer players are participating in a team sport. As such, they do not need to excel in both aerobic and anaerobic capacities, but must have a reasonably high performance level in all areas of physical performance [22]. This complex interplay of 'mixed' physical and metabolic performance abilities might be associated with a complex genetic profile, with different genetic variants, such as the *NOS3* -786T/C polymorphism playing a role on elite soccer players' status, either individually or in combination with other polymorphisms which are yet to be identified.

There are limitations in the curent study that must be kept in mind. Genetic association studies as the present one must be interpreted with caution since the possibility of false positive results attributable to chance cannot be dismissed. Genotype distributions vary among populations of different geographic and ethnic backgrounds. In this sense, we cannot exclude the possibility that our results are unique to the specific Spanish population of elite soccer players used in our study. On the other hand, *NOS3* -786 T/C genotypes did not meet HWE in power and endurance athletes, which limits the possibility of drawing solid conclusions from the comparison between soccer players and the other athletes. It has been recently recognized that in case-control studies (as our current study), the deviation from the HWE in groups other than the control group does not invalidate statistical analyses, as opposed to genetic cohort studies (in which a single group is studied) [1]. Finally, further research is necessary to determine the possible association between *NOS3* -786 T/C polymorphism and physiological indicators of physical performance in soccer, e.g. VO_2max or ability to perform repeated sprints.

In conclusion, the -786CC genotype and the -786C allele are associated with elite soccer player's status compared to elite endurance athletes, elite power athletes, and controls. More research is needed in elite soccer players of a different geographic/ethnic background in order to replicate the results of the present study. Mechanistic approaches are also needed to explain the putative role of the C allele in endurance or other exercise-related phenotypes, as well as in soccer performance.

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Legend to Figure:

Figure 1. Allele frequencies of the *NOS3* -786 T/C polymorphism (rs2070744) in Spanish (Caucasian, all males) controls, elite soccer players, elite endurance athletes and elite power athletes.

Soccer vs. Controls: χ^2=10.832, P=0.001

Soccer vs. Endurance: χ^2=7.230, P=0.007

Soccer vs. Power: χ^2=24.993, P<0.001