The Endothelial Nitric Oxide Synthase Gene Variant rs2070744 in Turkish Elite Athletes

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Abstract
Aim: Genetic variations have been associated with physical performance. The Endothelial Nitric Oxide Synthase (eNOS) gene variants have been widely studied in this context. The aim of the present study is to compare the T-786C variant of the eNOS gene in Turkish elite athletes and control groups.

Material and Method: DNA samples were obtained from 52 elite athletes (45 male, 7 female) and 60 control subjects (49 male, 11 female). The T-786C variant of the eNOS gene was genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Results: TT, TC, CC genotypes of the T-786C variant of eNOS genotypes were observed in 40.0%, 48.3%, and 11.6% of control subjects and in 55.7%, 30.7% and 13.4% of elite athletes, respectively. There was not any statistically significant difference in genotype and allele frequencies of T-786C of the eNOS between the elite athlete and the control groups (p>0.05).

Discussion: The present study demonstrated that the T-786C variant of the eNOS gene is not associated with study population but larger sample analyses are needed in different groups of elite athletes in order to substantiate these findings.

Keywords
Nitric Oxide Synthase; the T-786C; Variant; Elite Athletes
Introduction
An elite athlete is defined as the person who has competed at a national or international level in a given sport [1]. In the past decade, the idea that genetic traits bear a strong association with physical performance has been widely accepted. Researchers are now focusing on investigation of the exact genetic profiles that contribute to sport performance and they are trying to determine the underlying mechanisms that play a role in specific fields of elite athletic performance.

Nitric oxide (NO) affects the control of skeletal muscle function, increases skeletal muscle glucose uptake during exercise and enhances mitochondrial ATP production. All of these processes modulate muscle strength [2]. NO is synthesized from L-arginine by the nitric oxide synthase (NOS) gene [3,4]. NOS family has three distinct isoforms: neuronal NOS (nNOS/NOS1), inducible NOS (iNOS/NOS2), and endothelial NOS (eNOS/NOS3) [4]. (Higashibata T). eNOS gene is one of the candidate genes to clarify human variations in health and exercise-related phenotypes. Human eNOS gene is localized on chromosome 7 (7q35-36) and contains 26 exons [5]. The T-786C variant (rs2070744), a thymidine to cytosine transition mutation, is present in the 5' flanking region of eNOS gene and decreases the promoter activity of eNOS, resulting in decrease of endothelial NO production [6]. It was reported that eNOS T-786C variant is related with resting blood pressure [7] and the blood pressure response to acute event of maximal aerobic exercise [8]. The C allele affects eNOS transcription, which is consistent with reduced NO production [9]. Because of the crucial role of NO in muscle adaptation to exercise, we compared the T-786C variant of eNOS gene in Turkish elite athletes and control groups in this study.

Material and Method
Patients
The study population consisted of 52 Turkish elite athletes (45 males and 7 females; aged between 14-30) and 60 unrelated controls (49 males and 11 females; aged between 18-34) who had no competitive sport experience. Subjects had similar ethnic backgrounds and they were all from the same geographic area. Written-informed consent was obtained from each participant before blood sampling. The study involving human subjects was approved by the Ethics Committee in Clinical Research of Gaziosmanpasa University (11-BADK-095) and the study was conducted in accordance with the Helsinki Declaration.

Genetic analysis
Genomic DNA was isolated from 2 mL venous blood according to kit procedure (Sigma, USA) and stored at -20°C. The eNOS T-786C variant was analyzed by polymerase chain reaction-restriction-restriction fragment length polymorphism (PCR-RFLP) methods using the following primers: 5'TGAGTGCTGGTGATCCCA-3' (forward) and 5'-GCCCTCACCC-CCACCCTGTC-3' (reverse). Method was carried out as described previously by Ordonez et al. [10]. Amplified products were digested byMspI enzyme. Two sets of digested products were formed as a result of allelic variation. One of these products was of 140 and 40 bp (-786T allele) and another set was of the 90, 50 and 40 bp (-786C allele) in length. Digested products were examined on a 2.5 % agarose gel stained with ethidium bromide.

Statistical Analysis
All statistical analyses were performed using computer SPSS Statistical Program Version 20.0 and Openepi 3.01 software package program. Continuous data were given as mean±SD (standart deviation) and min-max. Chi-square test was used to determine the significance of differences in the allele frequency and genotype distribution between the two study groups. Hardy-Weinberg equilibrium test was performed for both study groups. Odds ratio (OR) and 95% confidence intervals (CIs) were calculated. A p value < 0.5 was considered statistically significant.

Results
We genotyped 60 controls (average age: 23.12±5.59 years; 49 male, 11 female) and 52 elite athletes (average age: 21.52±4.46 years; 45 male, 7 female) for the T-786C variant of the eNOS gene. The demographic and clinical characteristics are presented Table I. In result of analysis eNOS T-786C variant, it was determined that there was no any statistical significant differences between Turkish elite athletes and unrelated control who had no competitive sport experience in terms of genotype and allele frequencies. The genotype and allele frequencies of the eNOS T-786C of both group are reported in Table II.

Table I. Clinical and demographics features of the control and elite athlete groups

| Characteristic                      | Control group | Study group |
|-------------------------------------|---------------|-------------|
| Gender, male/female, n (%)          | 49/11 (81.7/18.3) | 45/7 (86.5/13.5) |
| Age, mean ± SD, years               | 23.12±3.59     | 21.52±4.46  |
| Height, mean ± SD, years            | --            | 175.65±9.86 |
| Weight, mean ± SD, years            | --            | 70.75±11.94 |
| BMI, mean ± SD, years               | --            | 22.77±2.04  |
| Sport duration, mean ± SD, years    | --            | 8.62±4.33   |
| Smoking, Yes/No, n (%)              | --            | 12/40 (23.07/76.92) |
| Daily smoking, mean ± SD, piece     | --            | 2.27±4.42   |
| Alchool, Yes/No, n (%)              | --            | 6/46 (11.53/88.46) |
| Monthly alchool, mean ± SD, piece   | --            | 1.23±0.05   |
| Sport, Football/Basketball, n (%)   | --            | 38/14 (73.07/26.92) |
| Family history for sport, mean ± SD, person | -- | 4.08±0.86 |

BMI: Body mass index, SD: Standard deviation

Table II. The distribution of the T-786C variant of the eNOS genotypes and alleles in the athletes and controls.

| Gene       | n(52) | Controls (n=60) | p   | OR (CI 95%) |
|------------|-------|----------------|-----|-------------|
| eNOS Genotypes |
| TT         | 29 (55.7 %) | 24 (40 %) | >0.05 |
| TC         | 16 (30.7 %) | 29 (48.3 %) | >0.05 |
| CC         | 7 (13.4 %)  | 7 (11.6 %)  | >0.05 |
| TT+TC+CC   | 45.7     | 53.7         | 0.85 (0.26-2.71) |
| TT:TC+CC   | 23.29    | 36.24        | 0.53 (0.24-1.13) |
| Alleles    |
| T          | 74 (71.1 %) | 77 (64.1 %) | >0.05 |
| C          | 30 (28.8 %) | 43 (35.8 %) | 1.37 (0.78-2.43) |
Discussion
Elite athletes represent both endurance and power related traits. Sport performance is rather polygenic in nature because of the combined effect of hundreds of factors in genetic variance among individuals. Even though it is difficult to determine the accurate genetic factors of performance, in recent years, various gene variants have been analyzed to evaluate individual differences in elite athletes with phenotype-genotype association studies.

eNOS-derived NO is also known as "endothelium-derived relaxing factor" and has crucial functions, including regulation of vascular tone and regional blood flow, inhibition of vascular smooth muscle cell proliferation, modulation of leukocyte–endothelial interactions and thrombosis [11]. Furthermore, NO has an impact as a neurotransmitter in the brain by facilitating the conversion of soluble guanylyl cyclase to the second messenger molecule, cyclic guanine monophosphate (cGMP). cGMP relaxes the blood vessels following exercise, increasing blood flow to muscles following exercise to enhance glucose uptake [12].

Evidence from several studies suggests that NO also plays a role in human skeletal muscle glucose uptake during exercise [13], as well as in the regulation of oxygen consumption in the myocardium [14] and skeletal muscles [15].

eNOS gene has been considered as one of the candidate genes affecting high endurance performance due to the effects of NO on vascular tone. The functions of eNOS gene are confirmed with experimental studies using eNOS-knockout mice. It was reported that eNOS gene deficiency causes increased vascular smooth muscle cell proliferation in response to vessel injury [16], hypertension [17] increased diet-induced atherosclerosis [18] and decreased bleeding times [19].

eNOS gene has several polymorphic sites. In various studies, it was reported that eNOS T-786C variant to be associated with resting forearm blood flow [20] and the parasympathetic modulation response to aerobic exercise training [21] besides the differentiation of elite power from endurance athletes [22]. In vitro luciferase-based transcription analysis, it was shown that C allele of eNOS has a lower promoter activity than T allele [6].

In previous studies, it was reported that eNOS G894T variant was associated with physical performance [23] however in another study, it wasn’t found difference three variants of eNOS compared with controls [24]. When distribution of alleles is investigated, there are studies reporting that T allele is more abundant in athletes [25–27]. There are also studies suggesting that C allele is abundant [28]. In present study, we compared eNOS T-786C genotypes in elite athletes and control groups. The genotype and allele frequencies of eNOS T-786C variant showed no significant differences between athletes and control groups (p<0.05).

Conclusions
Although the present study does not imply any difference between the groups, larger sample analyses are needed in different groups of elite athletes to substantiate these findings.

Competing interests
The authors declare that they have no competing interests.

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