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

Elit Sporcularda Endotelyal Nitrik Oksit Sentaz Gen Varyantı rs2070744

eNOS and Turkish Elite Athletes

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Özet

Amaç: Genetik varyasyonlar fiziksel performans ile ilişkilidir. Endotelyal nitrik oksit sentaz (eNOS) gen varyantları bu alanda oldukça çok çalışılmıştır. Bu çalışmanın amacı Türk elit sporcu ve kontrol gruplarında eNOS T-786C varyantını karşılaştırmaktır. Gereç ve Yöntem: 52 elit sporcu (45 erkek, 7 kadın) ve 60 kontrolden (49 erkek, 11 kadın) DNA örnekleri elde edildi. eNOS T-786C varyantı polimeraz zincir reaksiyonu-restriksiyon parça fragman polimorfizmi (PZR-RFLP) metodu ile değerlendirildi. Bulgular: eNOS TT, TC, CC genotipleri sırasıyla kontrol grubunda %40,0, 48,3 ve 11,6 olarak belirlenirken, hasta grubunda %55,7, 30,7 ve 13,4 olarak saptandı. eNOS T-786G genotip dağılımı ve allel sıklıklarında elit sporcu ve kontrol grupları arasında istatistiksel olarak belirgin fark yoktu (p>0.05). Tartışma: Sunulan çalışmada eNOS geni T-786C varyantı ile çalışma popülasyonu arasında anlamlı bir ilişki saptanmamıştır. Ancak bulgularımızı doğrulamak için farklı elit atlet gruplarında çalışmaların yapıl¬masına ihtiyaç duyulmaktadır.

Anahtar Kelimeler

Nitrik Oksit Sentaz; T-786C; Varyant; Elit Sporcu

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 gene 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

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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. A 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 Gaziosmanpaşa University (11-BADK-095) and the study was conducted in accord with the Helsinki Declaration.

Genetic analysis

Genomic DNA was isolated from 2 mL venous blood according to kit procedure (Sigma, USA) and stored at - 20oC. The eNOS T-786C variant was analyzed by polymerase chain reaction-restriction-restriction fragment lenght polymorphism (PCR-RFLP) methods using the following primers: 5'-TGGAGAGTGCTGGTGTACCCCA-3' (forward) and 5'-GCCTCCACCCCCACCCTGTC-3' (reverse). Method was carried out as described previously by Ordenez et al. [10]. Amplified products were digested by MspI 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±3.59 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 allel 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±4.05
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 %)		
TC	16 (30.7 %)	29 (48.3 %)	>0.05	
CC	7 (13.4 %)	7 (11.6 %)		
TT+TC:CC	45:7	53:7	>0.05	0.85 (0.26-2.71)
TT:TC+CC	23:29	36:24	>0.05	0.53 (0.24-1.13)
Alleles				
T	74 (71.1 %)	77 (64.1 %)	>0.05	1.37 (0.78-2.43)
C	30 (28.8 %)	43 (35.8 %)		

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 "endothelial-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 guanisine 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 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.

References

1. Macarthur DG, North KN. Genes and human elite athletic performance. Hum Genet 2005;116: 331–339
2. Gao Y. The multiple actions of NO. Pflugers Arch 2010; 459(6): 829-39.
3. Ozturk E, Balat O, Pehlivan S, Ugur MG, Ozcan C, Sever T, et al. Endothelial nitric oxide synthase gene polymorphisms in preeclampsia with or without eclampsia in a Turkish population. J Obstet Gynaecol Res 2011; 37(12): 1778-83.
4. Higashibata T, Hamajima N, Naito M, Kawai S, Yin G, Suzuki S, et al. eNOS genotype modifies the effect of leisure-time physical activity on serum triglyceride levels in a Japanese population. Lipids Health Dis 2012; 11:150.
5. Kara N, Senturk N, Gunes SO, Bagci H, Yigit S, Turanli AY. Lack of evidence for association between endothelial nitric oxide synthase gene polymorphism (glu-298asp) with Behçet's disease in the Turkish population. Arch Dermatol Res 2006; 297(10): 468-71.
6. Nakayama M, Yasue H, Yoshimura M, Shimasaki Y, Kugiyama K, Ogawa H, et al. T-786-->C mutation in the 5'-flanking region of the endothelial nitric oxide synthase gene is associated with coronary spasm. Circulation 1999; 99(22): 2864-70.
7. Augeri AL, Tsongalis GJ, Van Heest JL, Maresh CM, Thompson PD, Pescatello LS. The endothelial nitric oxide synthase -786 T>C polymorphism and the exercise-induced blood pressure and nitric oxide responses among men with elevated blood pressure. Atherosclerosis 2009 ; 204(2): e28-34.
8. Olson KM Augeri AL, Seip RL, Tsongalis GJ, Thompson PD, Pescatello LS. Correlates of endothelial function and the peak systolic blood pressure response to a graded maximal exercise test. Atherosclerosis 2012; 222(1): 202-7.
9. Dengel DR, Brown MD, Ferrell RE, Reynolds TH, Supiano MA. A preliminary study on T-786C endothelial nitric oxide synthase gene and renal hemodynamic and blood pressure responses to dietary sodium. Physiol Res 2007; 56(4): 393-401.
10. Ordóñez AJ, Carreira JM, Franco AG, Sánchez LM, Alvarez MV, García EC. Two expressive polymorphisms on the endothelial nitric oxide synthase gene (intron4, 27 bp repeat and -786 T/C) and the venous thromboembolism.Thromb Res 2000; 99(6):563-6.
11. Huang PL. eNOS, metabolic syndrome and cardiovascular disease.Trends Endocrinol Metab 2009; 20(6):295-302.
12. Pellinger TK, Simmons GH, Maclean DA, Halliwill JR. Local histamine H(1-) and H(2)-receptor blockade reduces postexercise skeletal muscle interstitial glucose concentrations in humans. Appl Physiol Nutr Metab 2010; 35(5):617-26
13. McConell GK, Kingwell BA. Does nitric oxide regulate skeletal muscle glucose uptake during exercise? Exerc Sport Sci Rev 2006; 34(1):36-41.
14. Loke KE, Laycock SK, Mital S, Wolin MS, Bernstein R, Oz M, et al. Nitric oxide modulates mitochondrial respiration in failing human heart. Circulation 1999; 100(12):1291-7.
15. Wilkerson DP, Campbell IT, Jones AM. Influence of nitric oxide synthase inhibition on pulmonary O2 uptake kinetics during supra-maximal exercise in humans. J Physiol 2004; 561(Pt 2):623-35.
16. Moroi M, Zhang L, Yasuda T, Virmani R, Gold HK, Fishman MC, et al. Interaction of genetic deficiency of endothelial nitric oxide, gender, and pregnancy in vascular response to injury in mice. J Clin Invest 1998; 101(6):1225-32.
17. Huang PL, Huang Z, Mashimo H, Bloch KD, Moskowitz MA, Bevan JA, et al. Hypertension in mice lacking the gene for endothelial nitric oxide synthase. Nature 1995; 377(6546): 239-42.
18. Kuhlencordt PJ Gyurko R, Han F, Scherrer-Crosbie M, Aretz TH, Hajjar R, et al. Accelerated atherosclerosis, aortic aneurysm formation, and ischemic heart disease in apolipoprotein E/endothelial nitric oxide synthase double-knockout mice. Circulation 2001 ; 104(4): 448-54.
19. Freedman JE, Sauter R, Battinelli EM, Ault K, Knowles C, Huang PL, et al. Deficient platelet-derived nitric oxide and enhanced hemostasis in mice lacking the NOSIII gene. Circ Res 1999; 84(12): 1416-21.
20. Data SA, Roltsch MH, Hand B, Ferrell RE, Park JJ, Brown MD. eNOS T-786C genotype, physical activity, and peak forearm blood flow in females. Med Sci Sports Exerc 2003; 35(12): 1991-7.
21. Silva BM, Neves FJ, Negrão MV, Alves CR, Dias RG, Alves GB, et al. Endothelial nitric oxide synthase polymorphisms and adaptation of parasympathetic modulation to exercise training. Med Sci Sports Exerc 2011; 43(9): 1611-8.
22. Gómez-Gallego F, Ruiz JR, Buxens A, Altmäe S, Artieda M, Santiago C, et al. Are elite endurance athletes genetically predisposed to lower disease risk? Physiol Genomics 2010; 41(1): 82-90.
23. Saunders CJ, Xenophontos SL, Cariolou MA, Anastassiades LC, Noakes TD, Collins M. The bradykinin beta 2 receptor (BDKRB2) and endothelial nitric oxide synthase 3 (NOS3) genes and endurance performance during Ironman Triathlons. Hum Mol Genet 2006; 15(6): 979-87.
24. Wolfarth B, Rankinen T, Mühlbauer S, Ducke M, Rauramaa R, Boulay MR, et al. Endothelial nitric oxide synthase gene polymorphism and elite endurance athlete status: the Genathlete study. Scand J Med Sci Sports 2008; 18(4): 485-90.
25. Gómez-Gallego F, Ruiz JR, Buxens A, Artieda M, Arteta D, Santiago C, et al. The -786 T/C polymorphism of the NOS3 gene is associated with elite performance in power sports. Eur J Appl Physiol 2009; 107(5): 565-9.
26. Drozdovs'ka SB, Lysenko OM, Dosenko VIe, Il'ïn VM, Moïbenko OO. [T(-786) --> C-polymorphism of the endothelial nitric oxide synthase promoter gene (eNOS) and exercise performance in sport]. Fiziol Zh 2013; 59(6): 63-71.
27. Sessa F, Chetta M, Petito A, Franzetti M, Bafunno V, Pisanelli D, et al. Gene polymorphisms and sport attitude in Italian athletes.Genet Test Mol Biomarkers 2011; 15(4): 285-90.

28. Eynon N, Ruiz JR, Yvert T, Santiago C, Gómez-Gallego F, Lucia A, et al. The C allele in NOS3 -786 T/C polymorphism is associated with elite soccer player's status. Int J Sports Med 2012; 33(7): 521-4.

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