

Do G894T Polymorphisms of Endothelial Nitric Oxide Synthase 3 (NOS3) Influence Endurance Phenotypes?

by

Paweł Ciężczyk¹, Marek Sawczuk², Agnieszka Maciejewska²,
Nijole Jascaniene³, Jerzy Eider¹

Endothelial nitric oxide (NO) synthase gene (NOS3) is taken into account as one of the main regulators of blood pressure and basal vascular dilation - two main factors found to be limiting for endurance performance.

We compared genotypic and allelic frequencies of the NOS3 G894T polymorphism in two groups of men of the same Caucasian descent: elite endurance athletes (rowers; n=63) and sedentary controls (n=160).

We have not found any statistical difference in G894T genotype and allele frequencies in endurance orientated athletes compared to sedentary controls. The difference in G allele frequency between the rowers and controls did not reach statistical significance (73.5% vs. 67.2%, $P = 0.307$), similar to genotype distribution amongst the rowers (58.7% GG; 39.4% GT; 6.4% TT) compared to controls (43.7% GG; 46.9% GT; 9.4% TT) ($P=0.129$).

In summary, our results are in contradiction to the hypothesis that NOS3 G894T polymorphism is associated with the physical performance status in rowing. Of course, our findings do not mean that other polymorphisms in NOS3 gene do not have any beneficial effect on performance parameters, but to confirm that hypothesis, we need further studies.

Key words: NOS3, genotype, rowing, endurance performance

Introduction

Exercising muscles require an increased delivery of oxygen and metabolic substrates (Wolfarth et al., 2008). This is why skeletal muscle blood flow capacity and altered control of total and regional muscle blood flow may be considered one of the main factors that influence endurance performance.

One of the key substances that influences blood pressure and basal vascular tone is nitric oxide (NO) (Quyyumi et al., 1995; Kimura et al., 2003). This con-

clusion is supported by Massion et al. (2003), who showed that NO in vascular endothelium regulates vasodilatation, and eventually blood pressure. Harrison and Cai (2003) have also reported that NO physiologically regulates basal vascular tone and vascular function by inhibiting platelet and leukocyte adhesion, and by affecting the growth of smooth muscle cells.

In addition, there is strong evidence suggesting that NO is also involved in human skeletal muscle glucose uptake (McConnell and Kingwell, 2006), as well as the modulation of oxygen consumption in

¹ - University of Szczecin, Institute of Physical Culture, Poland

² - University of Szczecin, Department of Genetics, Poland

³ - Vilnius Pedagogical University, Studentu St. 39, LT-08106 Vilnius, Lithuania

skeletal muscles (Wilkerson et al., 2004). Furthermore, Shen et al. (1995) suggests that NO regulates mitochondrial metabolism to optimize the ratio between oxygen consumption and energy production.

Nitric oxide is generated by endothelial NO synthase (eNOS), a product of the NOS3 gene (Rankinen et al., 2000). This gene is situated on the 7th chromosome in locus 7q36 and consists of 23,530 nucleotides (Robinson et al., 1994). Several polymorphic sites have been identified within the NOS3 gene (i.e., Glu298Asp (G894T or rs1799983) in exon 7, microsatellite (CA)_n repeats in intron 13, and 27-bp repeats in intron 4 (4B/4A) variations (Ahmetov and Rogozkin, 2009) and others. In the context of sport research, the most analyzed is the SNP G894T missense type (Glu298Asp) polymorphism within exon 7.

The G894T polymorphism is associated with exercise related phenotype traits (e.g., cardiovascular responses to exertion in the non-elite athletic population (Hang et al., 2006; Gomez et al., 2009) and VO₂ kinetics under heavy-intensity exercise (Jones et al., 2004).

Based on knowledge about the role of NOS3 G894T polymorphism in the regulation of endothelial function and blood pressure, we postulated that the genetic polymorphism of NOS3 could also influence elite endurance performance status. This hypothesis seems to be supported by Hand et al. (2006), who showed an association of the NOS3 G894T genotype with different exercise phenotypes, including heart rate, in post-menopausal women. Additionally, Sanders et al. (2006) found a significant correlation between NOS3 G894T polymorphism and actual performance during Ironman Triathlons.

According to Wang et al. (2000), the T allele of missense G894T polymorphism within NOS3 gene may be associated with reduced eNOS activity and basal NO production (and finally blood pressure). If this conclusion is valid, the GG genotype should, in theory, be more beneficial for endurance athletes than any other genotype at this polymorphic locus, as it may improve the endurance coefficients in sports people through an increase in the aerobic capacity of muscles.

The aim of this study is to perform preliminary studies to analyze the possible importance of the NOS3 gene polymorphisms in elite Polish rowers and sedentary individuals representing the possible relationships with genotype and physical performance.

Materials and methods

Ethics Committee

The Pomeranian Medical University Ethics Committee approved the study and written informed consent was obtained from each participant.

Subjects and controls

Sixty-three male Polish rowers (former and active athletes) of national competitive level were recruited for this study. Thirty-four were elite rowers, being national representatives, including world champions, with no less than ten years experience participating in sport. Additionally, 9 of the elite rowers were European Championship medalists, 7 of them were world championship medalist and 14 of them were Olympic Games medalists. The second group of recruited athletes (n=29) were non-elite rowers containing young Polish rowers (aged 18-22) who were regional competitors.

For controls, samples were prepared from 160 unrelated volunteers (male students from the University of Szczecin aged between 19-23yrs). The athletes and controls were all Caucasian to ensure no likely racial gene skew and to overcome any potential problems of population stratification.

Genotyping

The buccal cells donated by the subjects were collected in Resuspension Solution (Sigma, Germany) with use of Sterile Foam Tipped Applicators (Puritan, USA). DNA was extracted from the buccal cells using GenElute Mammalian Genomic DNA Miniprep Kit (Sigma, Germany) according to the producer protocol.

NOS3 Glu298Asp SNP (SNP 15: rs1799983): (PCR), followed by digestion with Ban II was performed. The PCR was performed in standard buffer and each 20- μ L PCR test contained 100 ng genomic DNA, 0.2 mmol/L each primer, 200 mM each dNTPs and 0.5U Taq polymerase. The reactions were incubated at 94 °C for 3 min, 60 °C for 1 min and 72 °C for 1 min, followed by 35 cycles of 94 °C for 30 s, annealing at 60 °C for 30 s and extension at 72 °C for 45 s and finally one cycle of 72 °C for 10 min. The PCR product was digested with 5U at 37 °C for 4 h. The resulting fragments were separated on 2.5% acrylamide gel and visualized under UV light after ethidium bromide staining.

Table 1

Genotype and allele frequencies of NOS3 G894T. This data is presented as absolute and relative values (within parentheses). *P* value corresponds to comparisons in genotype and allele frequencies within groups.

Group	N	NOS3 genotype			<i>P</i>	NOS3 allele		<i>P</i>
		GG	GT	TT		G	T	
All rowers	63	37 (58.7%)	22 (34.9%)	4 (6.4%)	0.129	96 (76.2%)	30 (23.8%)	0.062
Elite rowers	34	19 (55.9%)	12 (35.3%)	3 (8.8%)	0.417	50 (73.5%)	18 (26.5%)	0.307
Non-elite rowers	29	18 (62.1%)	10 (34.5%)	1 (3.4%)	0.163	46 (79.3%)	12 (20.7%)	0.066
Controls	160	70 (43.7%)	75 (46.9%)	15 (9.4%)	-	215 (67.2%)	105 (32.8%)	-

Statistical analysis

Genotype distribution and allele frequencies between the groups of athletes and controls were compared and significance was assessed by χ^2 test using STATISTICA 8 statistical software. *P* values of < 0.05 were considered statistically significant.

Results

NOS3 genotype distributions amongst subjects and controls were in Hardy-Weinberg equilibrium, making selection bias less likely. Genotype distribution results of the control group (GG-43.75%; GT-46.88%; TT-9.38%) were similar to those reported in previous studies on Caucasian populations (Wolfarth et al., 2008; Allanore et al., 2004). The distributions of the NOS3 genotypes and alleles are given in Table 1.

Allele frequencies of NOS3 G894T did not differ between elite rowers and sedentary controls (73.5% vs. 67.2%, $p = 0.307$). As shown in Table 1, the difference in allele frequency between non-elite rowers and controls also did not reach statistical significance, however in this case the disproportion was visibly bigger (79.5% vs. 67.2%, $p=0.066$). The same trend of a higher, yet not significant allele proportion, was noted over the whole cohort of athletes, compared to sedentary controls (76.2% vs. 67.2%, $p=0.062$).

NOS3 GG genotype was not more prevalent in the group of endurance athletes than in the controls, as was theoretically suspected. The genotype distribution (Fig. 1) amongst the whole cohort of rowers (58.7% GG; 39.4% GT; 6.4% TT) was not significantly different to that amongst sedentary controls (43.7% GG; 46.9% GT; 9.4% TT) ($p=0.129$). More importantly, in the case of the elite rowers, the proportion of NOS G894T genotype was even more similar to sedentary controls ($p=0.417$), clearly opposite to the hypothesis mentioned in the introduction of this article.

Discussion

The articles concerning the NOS3 gene in a sport context are still unique. Moreover, till now any hypothesis referring the role of this gene in physical performance status has not been clearly proven.

According to the Bray et al. (2009), the NOS3 gene is one of the candidate genes explaining individual variations in health and exercise capacity-related phenotypes because it encodes the eNOS enzyme, which catalyses the synthesis of nitric oxide (NO). Since the discovery of NO in the early 1980s, this molecule was associated as playing a pivotal role in the regulation of important systems and functions in the human organism. As was mentioned in the introduction of this article, NO regulates blood pressure (Massion et al., 2003) and basal vascular tone (Quyyumi et al., 1995), which determinate the blood supply to working muscles (Heydemann and McNally, 2009). NO is also involved in the modulation of oxygen consumption in skeletal muscle (Wilkerson et al., 2004) and glucose uptake into working muscle fibers (McConnell and Kingwell, 2006). Additionally, nitric oxide (NO) plays an important role in cardioprotection (Otani 2009) and myocardial respiration (Loke et al., 1999). Finally,

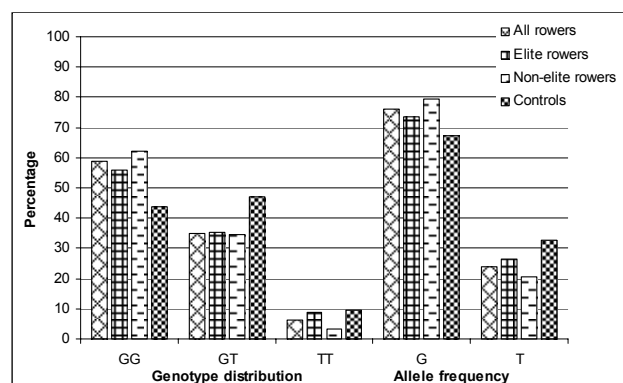


Figure 1
The NOS3 G894T genotype and allele frequencies in all groups

there are suggestions that NO regulates mitochondrial metabolism to optimize the ratio between oxygen consumption and energy production (Shen et al., 1995). In this context, it is worth mentioning investigations by Jones et al. (2004), which showed that pharmacological NOS inhibition led to significant speeding of VO₂ kinetics.

Few reports have shown that the NOS3 G894T genotypes are associated with predispositions to physical performance, including top endurance-orientated athletes. Sanders et al. (2006) found a correlation between NOS3 G894T polymorphism and actual performance during Ironman Triathlons. This finding seems to be supported by Hand et al. (2006), who also showed an association of the NOS3 G894T genotype with different exercise phenotypes. On the other hand, Wolfarth et al. (2008) found no association between NOS3 G894T variation and elite endurance performance, although it did show a significant association between VO₂max and genetic NOS3 variation.

Our results are in opposition to observations of Sanders et al. (2006) and Hand et al. (2006). We have not found any statistical difference in G894T genotype and allele frequencies in endurance orientated athletes compare to sedentary controls. Summarizing, we have reached a similar conclusion to Wolfarth et al. (2008). These findings seem to be additionally supported by Rankinen et al. (2000), who stated that there is no data available on associations between the NOS3 G894T polymorphism and plasma NO metabolite levels.

On the other hand, the same author had a hypothesis that, although NOS3 G894T polymorphism doesn't play a role in short-term response to a single bout of exercise, it may be a significant factor in the long-term adaptation of hemodynamic phenotypes to endurance training (Rankinen et al., 2000). In this context, the G allele of NOS3 G894T polymorphism could be a marker for sedentary individuals, who are most likely to benefit from endurance training in terms of reduction in the hemodynamic load.

The role of NOS3 gene in sport status seems to be still unclear. Even if NOS3 G894T polymorphism is not correlated with a predisposition to sport performance, there are several polymorphisms in the NOS3 gene

under the hypothesis that these polymorphisms may change the functional properties of NO, and hereby modify the different links by which NO is thought to influence endurance capacity (Wolfarth et al., 2008). Also, other reports showed the association between DNA sequence variations at NOS3 locus contributes significantly to the plasma levels of NO metabolites (Tsukada et al., 1998; Gomez-Gallego et al., 2009). All of the mentioned reports appear to require confirmation by further scientific works, taking into account the physical performance parameters.

Another aspect of the NOS3 polymorphism that warrants further studies is the possible interaction with other genetic and environmental factors. Sanders et al. (2006) pointed out that the effect of the genotype GG, advantageous for endurance performance, appeared only in connection with the genotype (-9/-9) of the gene BDKRB2. In other combinations of genotypes of both genes (NOS3 and BDKRB2), the genotype GG did not show any positive correlation with an increase in sport endurance, which suggests that although the gene NOS3 may be associated with efficiency and endurance parameters, the affirmative effect of the genotype GG is masked by the genotype +9/-9 BDKRB2. Additional questions are also associated with the correlations of NOS3 gene with the muscarinic receptor type 2 (m2) gene and voltage-gated potassium channel subfamily H, member 2 (KCNH2) gene (Wolfarth et al., 2008). It is suspected that both these genes might be, in conditions of further linkage, disequilibrium with some functional variants that lay in the NOS3 gene (Spina et al., 1997; Borggreffe et al., 2005). Rankinen et al. (2000) has also pointed out that coding the NOS has several co-factors, such as tetrahydrobiopterin and calmodulin, which are necessary for the optimal function of the enzyme. The genes that encode these proteins are also potential candidates themselves, because of their interactions with NOS3 (Rankinen et al., 2000).

In conclusion, our results are contrary to the hypothesis that NOS3 G894T polymorphism is associated with physical performance status in rowing. Of course, our finding does not mean that other polymorphisms in NOS3 gene do not have any beneficial effect on performance parameters, but to confirm this hypothesis, we need further studies.

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Corresponding author

Paweł Ciężczyk

University of Szczecin, Institute of Physical Culture, Poland

40B, Piastów Str., blok 6, 71-065 Szczecin

Phone/Fax: +48 91 444 27 49

E-mail: ciezczyk@poczta.onet.pl