Short Communication

The *eNOS* Gene Polymorphism Does Not Have a Major Impact on Lipid Parameters and Premature Coronary Artery Disease in Slovene Men (Caucasians)

(*eNOS* 4a/b gene polymorphism / premature coronary artery disease)

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Abstract. eNOS affects the NO level in the blood vessel wall, and therefore *eNOS* might be considered as a candidate gene for CAD. In this cross-sectional case-control association study we tested the hypothesis whether the *eNOS* 4a/b gene polymorphism is a genetic marker for premature CAD in Slovene men. The *eNOS* 4a/b gene polymorphism was tested in 403 Slovene men: 215 cases with premature CAD and 188 subjects with no history of CAD. The frequency of 4a/b genotypes did not differ between patients and controls: in CAD patients the frequencies of the 4aa, 4ab, or 4bb genotype were 5.0%, 27.9%, or 67.1%, respectively, and in controls the genotype frequencies were 5.3%, 30.9%, or 63.8%, respectively. In this study the aa genotype of the *eNOS* 4a/b polymorphism was not associated with premature CAD (OR = 1, 95% CI 0.4–2.3, P = 0.9). Moreover, there were no differences in lipid parameters (total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides) between the subjects with the aa genotype and the subjects with the ab or bb genotype. In conclusion, we failed to demonstrate that the *eNOS* 4a/b gene polymorphism was a genetic marker for premature CAD in Slovene men.

Several candidate genes have been implicated so far in the pathogenesis of the atherothrombotic process on coronary arteries, i.e. myocardial infarction (MI), including the angiotensin-I converting enzyme gene, apoprotein E gene, oestrogen gene, thrombotic genes, and haemochromatosis gene (Petrovic et al., 2000; Petrovic and Peterlin, 2003a; Petrovic et al., 2003b; Petrovic, 2004; Zorc et al., 2004). Another candidate gene for coronary artery disease (CAD) is endothelial nitric oxide synthase (*eNOS*) that affects the nitric oxide (NO) level in the blood vessel wall (Wang et al., 1996). After the initial report of the association between the aa genotype of the *eNOS* 4a/b polymorphism and CAD, subsequent studies from Europe, Japan, and USA gave controversial results (Hibi et al., 1998; Hooper et al., 1999; Sigusch et al., 2000). Moreover, the frequencies of the *eNOS* gene polymorphism might differ not only from population to population, but also by gender within these populations (Hibi et al., 1998; Hooper et al., 1999; Sigusch et al., 2000). In this association study we tested the hypothesis whether the *eNOS* 4a/b polymorphism is a genetic marker for premature CAD in Slovene men.

Material and Methods

In this cross-sectional case-control study we enrolled 215 men with premature CAD (study group) and 188 healthy men (control group). The patients were managed for chest pain, and the diagnosis of CAD was confirmed by coronary angiography. The patients and control subjects came from independent families. The controls did not have a history of angina pectoris or MI, and they had a normal electrocardiogram. All the subjects enrolled in the study were Slovene. The research protocol was approved by the national medical ethics committee. The *eNOS* 4a/b gene polymorphism was analysed as described previously (Wang et al., 1996). Differences in mean values between CAD patients and control subjects were analysed by Student's t-test. The χ^2 test was used to compare discrete variables. Statistical analysis was performed using the SPSS program for Windows 98 version 12 (SPSS Inc., Chicago, IL).

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Abbreviations: CAD – coronary artery disease, eNOS – endothelial nitric oxide synthase, MI – myocardial infarction, NO – nitric oxide.

Table 1. Distribution of eNOS genotypes among CAD patients and controls

Genotype	CAD patients (%)	Controls (%)	OR (95 % CI) [1]	P
aa	11 (5.0)	10 (5.3)	1.0 (0.4-2.3)[2]	0.9
ab	60 (27.9)	58 (30.9)		
bb	144 (67.1)	120 (63.8)		

[1]Odds ratio (95% confidence interval), [2]OR (95% CI) and P-value for the recessive model (aa genotype vs. ab genotype plus bb genotype)

Results and Discussion

The genotype distribution of the *eNOS* 4a/b gene polymorphism in the CAD group and in the control group were compatible with Hardy-Weinberg expectations (CAD group: $\chi^2 = 1.9$, P = 0.16; control group: $\chi^2 = 0.72$, P = 0.40). In our study there were no differences in total cholesterol (5.98 mmol/l ± 0.77 mmol/l vs. 6.30 mmol/l ± 1.52 mmol/l; P = 0.6), LDL cholesterol (4.08 mmol/l ± 0.72 mmol/l vs. 4.15 mmol/l ± 1.37 mmol/l; P = 0.9), HDL cholesterol (1.12 mmol/l ± 0.18 mmol/l vs. 1.13 mmol/l ± 0.52 mmol/l; P = 0.9), and triglycerides (1.75 mmol/l ± 0.72 mmol/l vs. 2.32 mmol/l ± 1.68 mmol/l; P = 0.9) between the subjects with the aa genotype and the subjects with the ab or bb genotype. The results of this case-control study show that the aa genotype of the *eNOS* 4a/b gene polymorphism is not associated with premature CAD in Slovene men (OR = 1, 95% CI 0.4–2.3, P = 0.9) (Table 1). This finding differs from the one in the case-control study by Wang et al., who first reported the association between the aa genotype of the *eNOS* 4a/b gene polymorphism and CAD in Caucasian Australian patients (Wang et al., 1996). The association was later confirmed in Japanese, in African Americans and in Turkish population (Ichihara et al., 1998; Hooper et al., 1999; Cine et al., 2002). Our results, however, are in agreement with previous reports, viz. that the *eNOS* 4a/b polymorphism is not associated with severity of CAD (Yoon et al., 2000; Via et al., 2003; Letonja, 2004).

The frequency of the *eNOS* genotypes in our patients with CAD (5%, 27.9% and 67.1% for the aa, ab and bb genotype, respectively) was practically identical to the Turkish MI patients reported by Cine et al. (2002) (4.3%, 26.6%, 69.1% for the aa, ab, bb genotype), and to the Slovene MI female patients reported by Letonja (2004) (4%, 27.2% and 68.8% for the aa, ab and bb genotype, respectively) and differed from the report in Caucasian Australian patients (Wang et al., 1996) (1%, 32% and 67% for the aa, ab and bb genotype, respectively), and two Japanese reports (2%, 23%, 75% for the aa, ab, bb genotype, and 1.8%, 21.2%, 77.0% for the aa, ab, bb genotype) (Ichihara et al., 1998; Hibi et al., 1998). This demonstrates a genetic heterogeneity in various populations, therefore we consider this to be due to the population bias. Contrary to reports from Europe (Caucasians), Japan and USA (African Americans) we failed to demonstrate gender differences (male vs. female patients) in the frequencies of the *eNOS* gene polymorphism in the Slovene population ((Hibi et al., 1998; Hooper et al., 1999; Sigusch et al., 2000; Letonja, 2004). In our study we found the alleles of four and five repeats as previously described in Caucasian and Asian populations, and we did not find the alleles of two, three and six repeats found in African-American populations (Hooper et al., 1999). In conclusion, we failed to demonstrate that the *eNOS* 4a/b gene polymorphism was a genetic marker for premature CAD in Slovene men.

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