ACTA MEDICA SALINIANA

ISSN 0350-364X

Acta Med Sal 2009; 38 (1): 13-16

ORIGINAL PAPER

THE 4 A/B POLYMORPHISM OF THE ENOS GENE AS A MARKER FOR MYOCARDIAL INFARCTION IN TYPE 2 DIABETES

Ines CILENŠEK
Klemen PILIH
Danijel PETROVIČ

Institute of Histology and Embryology,
Medical Faculty
University of Ljubljana

Ljubljana, Slovenia

Received: 15.12.2008.
Accepted: 01.02.2009.

Correspondence to:
Danijel Petrovič
Institute of Histology and Embryology, Medical Faculty, University of Ljubljana,
Korytkova 2, Ljubljana 1000
Slovenia
tel.: +386 1 543 73 67

e-mail: Daniel.Petrovic@mf.uni-lj.si

ABSTRACT

Introduction: Endothelial nitric oxide synthase (eNOS) affects nitric oxide (NO) level in the blood vessel wall, and therefore eNOS might be considered as candidate gene for myocardial infarction (MI). To investigate the impact of genetic polymorphisms of eNOS on MI in Slovenian population with type 2 diabetes, we searched for the association between the 4a/b polymorphism of the eNOS gene and MI in subjects with type 2 diabetes.
Methods: One hundred and fifty nine subjects with type 2 diabetes and MI were compared to 290 diabetic subjects without coronary artery disease (CAD). Chi-square test was used to compare discrete variables, and continuous clinical data were compared by unpaired students t test.
Results: In this study the AA genotype of the eNOS 4a/b polymorphism was not associated with MI in diabetics (OR = 1.3, 95% CI 0.6-2.8, P = 0.5).
Conclusion: We failed to demonstrate that the 4a/b polymorphism of the eNOS gene was associated with MI in Slovene type 2 diabetic patients, therefore it may not be used as a genetic marker for MI in Slovene type 2 diabetic patients.

Keywords: *eNOS 4 a/b gene polymorphism, myocardial infarction, diabetes mellitus*

INTRODUCTION

In the last 20 years the traditional understanding of vascular endothelium as a semi-selective barrier to the diffusion of macromolecules from blood vessels to interstitial space has evolved dramatically.[1,2,3,4,5] Numerous additional functions of endothelium have been identified, one of them being the synthesis of nitric oxide (NO), which is involved in modification of blood pressure and vessel tone.[3,4,6] Not only has NO vasodilatating functions, it also inhibits leukocyte adhesion, platelet aggregation, vascular smooth muscle growth and oxidation of low-density lipoprotein (LDL) cholesterol.[1,5] These actions suggest that endothelial NO has an important artheroprotective role.[1,2,3,4,5,6]

Endothelial NO is synthesized by endothelial NO synthase (eNOS) which is constitutively expressed in the endothelium. The gene encoding eNOS is located on the chromosome 7q35-36 and contains 26 exons which span 21 kolobases.[7] A 27 base pair (bp) repeat in the intron 4 of the eNOS gene (eNOS 4a/b) was the first of the gene polimorphisms to be identified as a genetic marker for coronary arthery disease (CAD) after Wang and co-workers found a correlation between homozygosity for the eNOS 4a allele and increased risk for CAD in current and ex-smokers.[2] Many subsequent studies from Europe, USA and Japan gave contradictory results.[8,9]

Table 1. *Characteristics of patients with MI and controls*

Characteristics	MI group n (%)	Controls n (%)	p Value
Number	159	290	
Age (years)	59.2±11.2	66.5±10.2	<0.001
Male sex	103 (64.8)	133 (45.9)	<0.001
BMI (kg/m²)	28.8±3.6	27.9±4.5	0.03
Arterial hypertension	103 (64.8)	203 (70.0)	0.9
Smoking habit	53 (33.3)	43 (14.8)	<0.001
Diabetes duration (years)	21.6±7.4	17.8±8.4	0.003
Total cholesterol (mmol/l)	5.9±1.4	5.5±1.3	0.007
HDL cholesterol (mmol/l)	1.1±0.3	1.2±0.4	0.027
LDL cholesterol (mmol/l)	3.7±1.3	3.2±1.0	<0.001
Triglycerides (mmol/l)	2.4±1.4	2.5±1.7	0.6

To investigate the impact of genetic polymorphisms of eNOS on myocardial infarction (MI) in Slovenian population with type 2 diabetes, we searched for the association between the 4a/b polymorphism of the eNOS gene and MI in subjects with type 2 diabetes.

MATERIALS AND METHODS

The study population of this cross-sectional analysis consisted of 449 subjects with type 2 diabetes lasting more than 10 years: 159 with MI and 290 subjects in the control group with no history of CAD, no signs of ischemic changes on electrocardiogram and no ischemic changes during submaximal stress testing. The diagnosis of MI was made according to the criteria by World Health Organization. Patients with MI were included in the study 1-9 months after the acute event. The patients and control subjects came from independent families. The data and blood samples of age-matched controls were obtained from general practitioners. The controls did not have a history of angina pectoris or MI, and had a normal electrocardiogram. All the subjects enrolled in the study were Slovenes of Slavic origin. After informed consent was obtained from the patients and control subjects, a detailed interview was made. Arterial hypertension and cigarette smoking were defined as binary variables. Patients were classified as having type 2 diabetes according to the current American Diabetes Association criteria for the diagnosis and classification of diabetes Expert Committee.[10] Body mass index (BMI) was calculated as weight in kilograms divided by the height in square meters. Total cholesterol, low density lipoproteins (LDL), high density lipoproteins (HDL) and triglycerides were determined by standard biochemical methods.

The eNOS 4a/b gene polymorphism was analyzed as described previously.[2,11] Genotyping was performed by two researchers (K. P., D.P.), blinded for case or control status of the patients. Chi-square test was used to compare discrete variables.

Differences in mean values were analyzed by Student t-test. Chi-square test was used to compare discrete variables and to compare genotype distributions. Genotypic odds ratios for MI with 95% confidence intervals with two-tailed p-values were calculated by Chi-square test. Statistical analysis was performed using the SPSS program 16 for Windows (SPSS Inc. Illinois).

RESULTS

The characteristics of the cases and control subjects are listed in Table 1. The cases were younger, predominantly of male sex and had a higher incidence of cigarette smoking compared to the control group. Additionally they had higher total cholesterol and low-density lipoprotein cholesterol levels, higher body mass index, longer duration of type 2 diabetes, and slightly lower high-density lipoprotein cholesterol levels than the controls. There were no significant dif-

Table 2. *Distribution of eNOS genotypes/alleles in patients with myocardial infarction (cases) and in those without coronary artery disease (controls).*

Genotype/allele	Cases n (%)	Controls n (%)	P	Odds ratio (95% confidence interval)
AA genotype	11 (6.9)	16 (5.5)		
AB genotype	48 (30.2)	87 (30)	0.5[1]	1.3 (0.6-2.8)[1]
BB genotype	100 (62.9)	187 (64.5)		
Total	159	290		
a allele	70 (22)	119 (20.5)	0.6[2]	
b allele	248 (78)	461 (79.5)		

ferences in the incidence of hypertension and triglyceride levels between the cases and control subjects.

The eNOS genotype distribution in patients (MI group) and controls were compatible with Hardy-Weinberg expectations (Table 1; MI group $\chi 2 = 1.639$, $p = 0.2$; controls $\chi 2 = 1.865$, $p = 0.2$). In cross-sectional study we failed to demonstrate an association between the AA genotype of the 4a/b polymorphism of the eNOS gene and MI in patients with type 2 diabetes (OR = 1.3; 95% CI 0.6-2.8; p = 0.5) (Table 2).

DISCUSSION

Our study failed to demonstrate an association between the AA genotype of the 4a/b polymorphism of the eNOS gene and MI in the Slovene population with type 2 diabetes. The results of our study are in agreement with previous reports in Slovene population (Caucasians) in general population. Letonja reported in 2004 that the 4a/b polymorphism of the eNOS gene was not associated with severity of CAD in Slovene women.[12] Similarly, this polymorphism did not have a major impact on lipid parameters and premature CAD in Slovene men.[11] Our findings differ from the results of studies made on Caucasian Australian patients,[13] Japanese,[14] African Americans[9] and in Turkish population.[15]

The frequencies of the eNOS genotypes in our diabetic patients with CAD (6.9%, 30.2% and 62.9% for the AA, AB and BB genotype, respectively) were similar to the frequencies reported in Slovene men (5%, 27.9% and 67.1%),[11] Slovene women (4%, 27.2% and 69.1%)[12] and in Turkish MI patients (4.3%, 26.6% and 69.1%).[15] The frequencies of the eNOS genotypes in our diabetic patients with CAD, on the other hand, differed from the frequencies reported in Caucasians from Australia (1%, 32% and 67%),[13] and Japanese (2%, 23% and 75%).[14] Compared to the frequency of the eNOS genotype distribution in our control group (5.5%, 30% and 64.5% for the aa, ab and bb genotype, respectively) it can be assumed that genetic heterogeneity among different populations might be the cause of different affect of the 4a/b polymorphism of the eNOS gene on the prevalence of CAD.

We presume that the lack of an association between the 4a/b polymorphism of the eNOS gene and MI in our study may be due to a multifactorial nature of the MI. Besides, controversial results of association studies may be explained by differences in study design or by genetic heterogeneity within and between the populations from which the samples were derived.

In conclusion, we failed to demonstrate that the 4a/b polymorphism of the eNOS gene was associated with MI in Slovene type 2 diabetic patients, therefore it may not be used as a genetic marker for MI in Slovene type 2 diabetic patients.

REFERENCES

1. Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev 1991; 43: 109-42.
2. Wang Y, Mardsen PA. Nitric oxide synthases: biochemical and molecular regulation. Curr Opin Nephrol Hypertens 1995; 4: 12-22.
3. Vallance P, Collier J, Moncada S. Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. Lancet 1989; 2: 997-1000.
4. Yao S-K, Ober JC, Krishnaswami A, Ferguson JJ, Anderson V, Golino P, Buja LM, Willerson JT. Endogenous nitric oxide protects against platelet aggregation and cyclic flow variations in stenosed and endothelium-injured arteries. Circulation 1992; 86: 1302-9.

5. Kubes P, Suzuki M, Granger DN. Nitric oxide: an endogenous modulator of leukocyte adhesion. Proc Natl Acad Sci U S A 1991; 88: 4651-5.
6. Quyyumi AA, Dakak N, Andrevs NP, Husian S, Arora S, Gilligan DM, Panza JA, Canon RO III. Nitric oxide activity in human coronary circulation. J Clin Invest 1995; 95: 1747-5.
7. Mardsen PA, Heng HH, Scherer SW. Structure and chromosomal localization of the human constitutive endothelial nitric oxide synthase gene. J Biol Chem 1993; 268: 17478-88.
8. Hibi K, Ishigami T, Tamura K, Mizushima S, Nyui N, Fujita T, et al. Endothelial nitric oxide synthase gene polymorphism and acute myocardial infarction. Hypertension1998; 32: 521-62.
9. Hooper WC, Lally C, Austin H, Benson J, Dilley A, Wenger NK, Whitsett C, Rawlins P, Evatt BL. The relationship between polymorphism in the endothelial cell nitric oxide synthase gene and the platelet GPIIIa gene with myocardial infarction and venous thromboembolism in African Americans. Chest 1999; 116: 880-6.
10. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, Diabetes Care 20 (1997), 1183–97.
11. Milutinovic A, Hruskovicova H. The eNOS gene polymorphism does not have a major impact on lipid parameters and premature coronary artery disease in Slovene men (Caucasians). Folia Biol (Praha) 2005; 51: 47-9.
12. Letonja M. The eNOS gene polymorphism does not have a major impact on lipid parameters and premature coronary artery disease in Caucasian women. Acta Cardiol. 2004;59: 618-22.
13. Wang XL, Sim AS, Badenhop RF, McCredie RM, Wilcken DE. A smoking-dependent risk of coronary artery disease associated with a polymorphism of the endothelial nitric oxide synthase gene. Nat Med. 1996; 2: 41-5.
14. Ichihara S, Yamada Y, Fujimura T, Nakashima N, Yokota M. Association of a polymorphism of endothelial constitutive nitric oxide synthase gene with myocardial infarction in the Japanese population. Am J Cardiol. 1998; 81: 83-6.
15. Cine, N, Hatemi AC, Erginel-Unaltuna N. Association of a polymorphism of the ecNOS gene with myocardial infarction in a subgroup of Turkish MI patients. Clin Gene 2002; 61: 66-70.