

ORIGINAL PAPER

MANGANESE SUPEROXIDE DISMUTASE GENE POLYMORPHISM (V16A) IS NOT ASSOCIATED WITH MYOCARDIAL INFARCTION IN SLOVENE TYPE 2 DIABETIC PATIENTS

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ABSTRACT

Aim: to investigate an association between the V16A MnSOD gene polymorphism and myocardial infarction (MI) in Slovene type 2 diabetic patients.

Patients and methods: in this case-control cross-sectional study a relationship between the alanine/valine single nucleotide polymorphism (SNP) at amino acid sequence 16 of manganese superoxide dismutase (Mn-SOD) gene and myocardial infarction (MI) in Slovene type 2 diabetic patients was evaluated.

Results: the V16A polymorphism of the Mn-SOD gene was analysed in 449 subjects with type 2 diabetes lasting more than 10 years: 159 with MI (diabetics with MI) and 290 diabetics with no history of CAD. The VV genotype of the V16A Mn-SOD SNP was not associated with MI in Slovene patients with type 2 diabetes (OR = 0.9; 95 % CI = 0.6-1.5; P = 0.7).

Conclusion: the V16A Mn-SOD SNP might not be used as a genetic marker for MI in Slovene type 2 diabetic patients.

Keyword: *MnSOD V16A gene polymorphism, myocardial infarction, diabetes mellitus*

INTRODUCTION

Cardiovascular disease is the leading cause of premature death in type 2 diabetic patients. Patients with type 2 diabetes were reported to have 2-4-fold increased risk of coronary artery disease.¹ After a first cardiac event, nearly 50% of patients with diabetes may die within 1 year; one-half of them die before reaching the hospital.² This suggests that early detection of CAD and knowledge about the pathogenesis of CAD in diabetics can play an important part in reducing the morbidity and mortality of CAD in diabetic patients.

In previous years, biochemical and genetic markers have contributed to a better understanding of the underlying pathophysiology of acute coronary syndromes in patients with/without diabetes.³ Substan-

tial data indicate that oxidative stress may have an important role in the pathogenesis of acute myocardial infarction in diabetics and may also be involved in the development of different complications in diabetics.^{4,5} Oxidative stress is the result of an imbalance between the amount of reactive oxygen species (ROS) and the capacity of antioxidant defense systems.⁶ The most common ROS in the cell is the superoxide radical, which is produced during oxidative phosphorylation within mitochondria. A key enzyme in antioxidant defense systems is manganese mitochondrial superoxide dismutase (Mn-SOD), which catalyses the removal of superoxide radicals at the site of production, the matrix side of the inner mitochondrial membrane. Mn-SOD, an 80-kDa tetramer of 196 amino acid residues, is present in high concentration in liver, heart, kidney, and adrenal gland. Heart muscle is rich in Mn-SOD, which is located mainly in the mitochondria.⁷ A

Table 1. *Characteristics of diabetics with myocardial infarction (MI) and diabetics without coronary artery disease (CAD)*

Characteristics	Diabetics with MI N (%)	Diabetics without CAD 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/m ²)	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

number of polymorphisms in this sequence have been described, but only the A16V has been demonstrated to have a functional significance.⁸⁻¹⁰ In fact, the protein encoded by the Val (V) allele which disrupts the alpha-helix structure, is retained at the level of the mitochondrial inner membrane and has been associated with a 30-40% lower activity and increased susceptibility to oxidative stress¹⁰. The V16A polymorphism of the Mn-SOD gene may therefore cause inter-individual differences in Mn-SOD protein localization. Additionally, large interracial differences in the allele frequency have been reported.^{8, 10-13}

Few studies have been recently reported to demonstrate an association between the V16A polymorphism of the MnSOD gene and carotid atherosclerosis, obesity and cardiovascular risk.¹⁴⁻¹⁷ Additionally, a possible interaction between MnSOD, ox-LDL and type 2 diabetes was suggested.¹⁵

The aim of the present study was to investigate an association between the V16A MnSOD gene polymorphism and myocardial infarction (MI) in Slovene type 2 diabetic patients.

PATIENTS AND METHODS

The population of this cross-sectional association study consisted of 449 subjects with type 2 diabetes lasting more than 10 years: 159 with MI (diabetics with MI) and 290 diabetics with no history of CAD, no ischemic changes on electrocardiogram and no isch-

emic changes during submaximal stress testing (diabetics without CAD). 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. All the subjects enrolled in the study were Slovenes of Slavic origin. After informed consent was obtained from the subjects with diabetics, 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.¹⁸ 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 V16A polymorphism of the Mn-SOD gene was evaluated by RFLP, using the primers: P1: 5'-CAG CCC AGC CTG CGT AGA CGG -3' and P2: 5'-CTT GGC CAA CGC CTC CTG GTA CTT-3', and the BsaW1 restriction enzyme as described by Degoul et al.⁸

Genotyping was performed by two researchers (I.C., 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-

Table 2. Distribution of Mn-SOD genotypes/alleles in diabetics with MI and diabetics without CAD

Genotype/allele	Diabetics with MI n (%)	Diabetics without CAD n (%)	P	Odds ratio (95% confidence interval)
VV genotype	38 (23.9)	73 (25.2)	0.7 ¹	0.9 (0.6-1.5) ¹
AV genotype	80 (50.3)	142 (49)		
AA genotype	41 (25.8)	75 (25.9)		
Total	159	290		
V allele	156 (49.1)	288 (49.7)	0.9 ²	
A allele	162 (50.9)	292 (50.3)		

¹P-value and OR for recessive model (VV versus AV plus AA or aa versus ab plus bb)

²P-value for allele frequency

square test. Statistical analysis was performed using the SPSS program 17 for Windows (SPSS Inc. Illinois).

RESULTS

The characteristics of diabetics with MI and diabetics without CAD 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 LDL cholesterol levels, higher BMI, longer duration of type 2 diabetes, and lower HDL cholesterol levels than the controls (Table 1). There were no significant differences in the incidence of hypertension, and triglyceride levels between the cases and control subjects (Table 1).

The Mn-SOD genotype distribution in diabetics with MI and diabetics without CAD were compatible with Hardy-Weinberg expectations (Table 2). In this cross-sectional study the VV genotype of the V16A Mn-SOD SNP was not found to be associated with MI in patients with type 2 diabetes in the Slovene population (OR = 0.9; 95 % CI = 0.6-1.5; P = 0.7; Table 2).

DISCUSSION

This study analyzed the V16A polymorphism of the Mn-SOD gene as a potential genetic marker of MI in subjects with type 2 diabetes. Our study failed to demonstrate the V16A polymorphism of the Mn-SOD gene to be associated with MI in type 2 diabetics. To our knowledge, there are no prior studies which investigated whether the V16A polymorphism of the Mn-SOD gene is associated with MI in subjects with type 2 diabetes. Recently, however, our group found an associa-

tion of the V16A polymorphism of the Mn-SOD gene with diabetic retinopathy in Slovene type 2 diabetes patients. A significantly higher frequency of the VV genotype of the V16A polymorphism of the Mn-SOD gene was found in patients with diabetic retinopathy compared to those without diabetic retinopathy.¹⁹ Nakanishi et al. have recently reported that the V16A polymorphism of the Mn-SOD gene is associated with the development of type 2 diabetes in Japanese-Americans. Compared with alanine allele carriers, subjects with a valine allele homozygote showed significantly higher risk for developing diabetes after adjustment for age, gender, systolic blood pressure, total cholesterol, BMI, and homeostasis model assessment.²⁰

Table 3. Logistic regression analysis for the association with myocardial infarction among type 2 diabetic patients

Risk factor	OR (95% CI) ¹	P
Smoking habit	3.6 (2.1-6.2)	< 0.001
Sex	2.0 (1.2-3.2)	0.005
VV genotype ²	1.1 (0.7-1.5)	0.8

¹ Odds ratio (95 % confidence interval)

² V/A polymorphism of the MnSOD gene

Large inter-racial differences in the allele frequency have been reported so far.^{8, 11-13} The frequency of the V allele in general population is around 0.50 in Caucasians,^{11,21,22} 0.85 in healthy Japanese population,¹³ and 0.88 in healthy Korean population.¹² Similar percentages of the V allele have been reported in diabetics and in non-diabetics.^{11-13,21} Interestingly, however, the V allele frequency in Japanese and Korean subjects was remarkably higher than in Caucasians.^{11-13,21}

Our study had few limitations. Firstly, patients with MI were included in the study 1 to 9 months after the acute event and retrospective cross-sectional association studies are prone to survival bias. Secondly, the exclusion of CAD on the basis of a negative history of MI or angina pectoris, and absence of ischemic changes on the electrocardiogram and during exercise stress testing has certain disadvantages. It is not possible to rule out the possibility that a proportion of patients in the control group had asymptomatic CAD. Bacci and co-workers reported that 18% of asymptomatic diabetic patients with a negative result of exercise stress testing presented silent CAD with significant ($\geq 70\%$) angiographically documented coronary stenosis.²³ The lack of an association between the polymorphism of the MnSOD gene and MI in the present study may be due to the multifactorial nature of CAD. This negative result may also be attributable to survival bias, especially in diabetic patients with MI, which is associated with increased early mortality. For example, even after the first cardiac event, 50% of patients with diabetes may die within 1 year, and half of those who die do so before they reach the hospital.²⁴

The third limitation of our cross-sectional study was the difference in age and in sex distribution between diabetics with MI and diabetics without CAD, however cross-sectional studies are prone to such differences.

Further studies enrolling larger numbers of patients from different populations are needed to confirm our findings. Namely, genetic association studies are prone to beta statistical error and population-specific genotype effects, all of which make the results difficult to reproduce. In fact, the calculated power of our study for the Mn-SOD polymorphism was 70 % taking into account the size of our study sample, and the frequencies of risk allele in cases and controls.

We may conclude that we failed to confirm our hypothesis, that the V16A polymorphism of the Mn-SOD gene might be a risk factor for MI in the Slovene population (Caucasians) with type 2 diabetes.

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REFERENCES

1. Uusitupa MI, Niskanen LK, Siitonen O, Voutilainen E, Pyörälä K: Ten-year cardiovascular mortality in relation to risk factors and abnormalities in lipoprotein composition in type 2 (non-insulin-dependent) diabetic and non-diabetic subjects. *Diabetologia* 1993; 36: 1175-84.
2. Haffner SM: Coronary heart disease in patients with diabetes. *N Engl J Med* 2000; 342: 1040-2.
3. Lindahl B, Toss H, Siegbahn A, Venge P, Wallentin L: Markers of myocardial damage and inflammation in relation to long-term mortality in unstable coronary artery disease. FRISC Study Group. *Fragmin during Instability in Coronary Artery Disease*. *N Engl J Med* 2000; 343: 1139-47.
4. Jay D, Hitomi H, Griendling KK: Oxidative stress and diabetic cardiovascular complications. *Free Radic Biol Med* 2006; 40: 183-92.
5. Di Filippo C, Cuzzocrea S, Rossi F, Marfella R, D'Amico M: Oxidative stress as the leading cause of acute myocardial infarction in diabetics. *Cardiovasc Drug Rev* 2006; 24: 77-87.
6. Allen RG, Tresini M: Oxidative stress and gene regulation. *Free Radic Biol Med* 2000; 28: 463-99.
7. Usui A, Kato K, Tsuboi H, Sone T, Sassa H, Abe T: Concentration of Mn-superoxide dismutase in serum in acute myocardial infarction. *Clin Chem* 1991; 37: 458-61.
8. Degoul F, Sutton A, Mansouri A, Cepanec C, Degott C, Fromenty B, Beaugrand M, Valla D, Pessayre D: Homozygosity for alanine in the mitochondrial targeting sequence of superoxide dismutase and risk for severe alcoholic liver disease. *Gastroenterology* 2001; 120: 1468-74.
9. Shimoda-Matsubayashi S, Matsumine H, Kobayashi T, Nakagawa-Hattori Y, Shimizu Y, Mizuno Y: Structural dimorphism in the mitochondrial targeting sequence in the human manganese superoxide dismutase gene. A predictive evidence for conformational change to influence mitochondrial transport and a study of allelic association in Parkinson's disease. *Biochem Biophys Res Commun* 1996; 226: 561-5.
10. Sutton A, Imbert A, Igoudjil A, Descatoire V, Cazanave S, Pessayre D, Degoul F: The manganese superoxide dismutase Ala16Val dimorphism modulates both mitochondrial import and mRNA stability. *Pharmacogenet Genomics* 2005; 15: 311-9.
11. Chistyakov DA, Savost'yanov KV, Zotova EV, Nosikov VV: Polymorphisms in the Mn-SOD and EC-SOD genes and their relationship to diabetic neuropathy in type 1 diabetes mellitus. *BMC Med Genet* 2001; 2: 4.
12. Lee SJ, Choi MG: Association of manganese superoxide dismutase gene polymorphism (V16A) with diabetic macular edema in Korean type 2 diabetic patients. *Metabolism* 2006; 55: 1681-8.
13. Nomiya T, Tanaka Y, Piao L, Nagasaka K, Sakai K, Ogiwara T, Nakajima K, Watada H, Kawamori R: The polymorphism of manganese superoxide dismutase is associated with diabetic nephropathy in Japanese type 2 diabetic patients. *J Hum Genet* 2003; 48: 138-41.
14. Kakko S, Päivänsalo M, Koistinen P, Kesäniemi YA, Kinnula VL, Savolainen MJ: The signal sequence polymorphism of the MnSOD gene is associated with the degree of carotid atherosclerosis. *Atherosclerosis* 2003; 168: 147-52.
15. Gottlieb MG, Schwanke CH, Santos AF, Jobim PF, Müssel DP, da Cruz IB: Association among oxidized LDL levels, Mn-SOD, apolipoprotein E polymorphisms, and cardiovascular risk factors in a south Brazilian region population. *Genet Mol Res* 2005; 4: 691-703.
16. Montano MA, Barrio Lera JP, Gottlieb MG, Schwanke CH, da Rocha MI, Manica-Cattani MF, dos Santos GF, da Cruz IB: Association between manganese superoxide dismutase (MnSOD) gene polymorphism and elderly obesity. *Mol Cell Biochem* 2009; 328: 33-40.

17. Fujimoto H, Taguchi J, Imai Y, Ayabe S, Hashimoto H, Kobayashi H, Ogasawara K, Aizawa T, Yamakado M, Nagai R, Ohno M: Manganese superoxide dismutase polymorphism affects the oxidized low-density lipoprotein-induced apoptosis of macrophages and coronary artery disease. *Eur Heart J* 2008; 29: 1267-74.
18. 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* 1997; 20: 1183-97.
19. Petrovic MG, Cilensek I, Petrovic D: Manganese superoxide dismutase gene polymorphism (V16A) is associated with diabetic retinopathy in Slovene (Caucasians) type 2 diabetes patients. *Dis Markers* 2008; 24: 59-64.
20. Nakanishi S, Yamane K, Ohishi W, Nakashima R, Yoneda M, Nojima H, Watanabe H, Kohno N: Manganese superoxide dismutase Ala16Val polymorphism is associated with the development of type 2 diabetes in Japanese-Americans. *Diabetes Res Clin Pract* 2008; 81: 381-5.
21. Bergman M, Ahnström M, Palmebäck Wegman P, Wingren S: Polymorphism in the manganese superoxide dismutase (MnSOD) gene and risk of breast cancer in young women. *J Cancer Res Clin Oncol* 2005; 131: 439-44.
22. Valenti L, Conte D, Piperno A, Dongiovanni P, Fracanzani AL, Fraquelli M, Vergani A, Gianni C, Carmagnola L, Fargion S: The mitochondrial superoxide dismutase A16V polymorphism in the cardiomyopathy associated with hereditary haemochromatosis. *J Med Genet* 2004; 41: 946-50.
23. Bacci S, Villella M, Villella A, Langialonga T, Grilli M, Rauseo A, Mastroianno S, De Cosmo S, Fanelli R, Trischitta V: Screening for silent myocardial ischaemia in type 2 diabetic patients with additional atherogenic risk factors: applicability and accuracy of the exercise stress test. *Eur J Endocrinol* 2002; 147: 649-54.
24. Haffner SM: Coronary heart disease in patients with diabetes. *N Engl J Med* 2000; 342: 1040-2.