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The –429 T/C and –374 T/A Gene Polymorphisms of the Receptor of Advanced Glycation End Products Gene (RAGE) are not Risk Factors for Coronary Artery Disease in Slovene Population With Type 2 Diabetes

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A B S T R A C T

Receptor for advanced glycation end products (RAGE) plays a role in atherosclerosis in diabetics. There are two functional polymorphisms in the promoter of the RAGE gene (–429T/C and –374T/A). The aim of this study was to look for a relationship between the –429T/C and the –374T/A gene polymorphisms of the RAGE gene and the development of coronary artery disease (CAD) in the Slovene population with type 2 diabetes of duration longer than 10 years. One hundred and sixty-eight subjects with diabetes and CAD were compared to 241 diabetic subjects without CAD. The –429T/C and the –374T/A RAGE genotype distributions in patients with CAD (–429T/C: CC: 3%, TC: 31%, TT: 66.0%; –374T/A: AA: 7.7%, TA: 48.2%, TT: 44.1%) were not significantly different from those in patients without CAD (–429 T/C: CC: 1.7%, TC: 26.1%, TT: 72.2%; –374T/A: AA: 11.2%, TA: 43.2%, TT: 45.6%). Our study failed to demonstrate an association between either the –429T/C or the –374T/A gene polymorphism of the RAGE gene and CAD in the Slovene population with type 2 diabetes of duration longer than 10 years.

Key words: *RAGE; –429 T/C, –374 T/A, coronary artery disease, Caucasians, type 2 diabetes, genetic risk factors*

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Introduction

The prevalence of type 2 diabetes has been increasing worldwide due to increasing obesity and decreasing physical activity[1]. In people with type 2 diabetes the leading cause of death is coronary artery disease (CAD)[1]. Several candidate genes for CAD such as the angiotensin-1 converting enzyme (*ACE*) gene, plasminogen activator inhibitor gene, apoprotein E gene, apoprotein A1 gene, estrogen receptor gene, and the hemochromatosis gene have so far been tested in the Slovene population, whereas the receptor for advanced glycation end products (RAGE) is another candidate gene for CAD that has not been tested in the Slovene population yet[2–10]. So far, one the DD genotype of the insertion/deletion gene polymorphism of the *ACE* gene has been demonstrated to be a genetic risk factor for CAD in Slovene population.

The RAGE is normally expressed at low levels in the vasculature, whereas it is over-expressed in the vessel wall in the presence of vascular diseases[11]. The RAGE has been implicated in the development of vascular complications of diabetes by both *in vitro* and *in vivo* studies[12]. Evidence to implicate the RAGE is provided by the beneficial effect the soluble RAGE has on the development of vascular diseases[13], as well as the demonstration of increased expression in diseased vascular tissues of diabetic animal models and human subjects[14,15]. The hyperglycemic milieu in diabetes results in the accelerated formation of advanced glycation end products (AGEs); the effects of AGEs have been shown to be mediated via various cellular receptors (macrophage scavenger receptor, LOX-1, OST 48, and PRKCSH)[16]. One of the most important receptor is the RAGE[16,17]. Activation of the RAGE via AGEs stimulates proinflammatory and procoagulatory pathways leading to vascular dysfunction[12,18]. The RAGE, AGEs and proinflammatory ligands are implicated in the pathogenesis of atherosclerosis[12,18]. Two functional polymorphisms in the promoter of the RAGE gene (–429 T/C and –374 T/A) that may be important in the pathogenesis of diabetic vascular diseases have recently been described[19,20].

The aim of this study was to look for a relationship between the –429 T/C and –374 T/A gene polymorphisms of the RAGE and the development of CAD in the Slovene population with type 2 diabetes of duration longer than 10 years.

Materials and Methods

Patients

The study population of this cross-sectional case-control association study consisted of 409 diabetic subjects with type 2 diabetes of duration longer than 10 years. In the CAD group (168 cases) 130 cases with a history of myocardial infarction (MI) (the diagnosis of MI was made according to the criteria by World Health Organization) and 38 cases with angina pectoris confirmed by either abnormal stress testing or abnormal perfusion scan[21]. The patients and diabetic control subjects came from independent families. The data and blood samples of patients with CAD and diabetic control subjects were obtained from the Diabetic Outpatient Clinic and the Eye Clinic of the University Medical Centre Ljubljana, and from general practitioners. The diabetic control group consisted of 241 diabetics with no history of CAD, no signs of ischemic changes on electrocardiogram and no ischemic changes during submaximal stress testing. All the subjects enrolled in the study were Slovene. The research protocol was approved by the national medical ethics committee. After informed consent was obtained from the patients and diabetic control subjects, a detailed interview was made. The physician interviewed each patient and diabetic control subject about

the coronary risk factors (cigarette smoking, arterial hypertension, body weight and height). Smoking habit was defined as a daily intake of more than 5 cigarettes. Arterial hypertension was measured ambulatory, and was defined as a binary variable. Arterial hypertension was defined as systolic blood pressure higher than 140 mm Hg, diastolic blood pressure higher than 90 mm Hg, or both, at repeated measurements, or current use of antihypertensive agents for the confirmed diagnosis of arterial hypertension. Patients were classified as having type 2 diabetes according to the current American Diabetic Association criteria for the diagnosis and classification of diabetes[22].

Total cholesterol, low density lipoproteins (LDL), high density lipoproteins (HDL) and triglycerides were determined by standard biochemical methods[21]

Genotyping

The –429 T/C and –374 T/A gene polymorphisms of the RAGE gene were genotyped by PCR-RFLP, using primers: –429 T/C (5'-TTTCTTTCACGAAG[T/C]TCCAAACAGGTTTC-3') and –374 T/A (5'-ATGCAGGCCCAA[T/A]TGCACCCTTGCA GA-3') followed by *Alu* I and *Tsp* 509 I restriction[19]. Genotyping was done by two researchers (AMŽ, KS), blinded to the disease status of the subjects with type 2 diabetes.

Statistics

Differences in mean values were analyzed by unpaired Student's t-test and presented as means ± standard deviation (SD). Chi-square test was used to compare discrete variables and genotype distributions. Genotypic odds ratios (OR) for CAD with 95% confidence intervals with two-tailed *p* values were calculated by multiple logistic regression analysis adjusted for cardiovascular risk factors (smoking, sex, LDL cholesterol, diastolic blood pressure, duration of diabetes, body mass index (BMI), –374 T/A gene polymorphism, –429 T/C gene polymorphism, age). Statistical analysis was performed using the SPSS program for Windows version 11 (SPSS Inc. Illinois).

Results

One hundred and sixty-eight diabetics with CAD were compared with 241 diabetics without CAD. The characteristics of the cases and control subjects are listed in Table 1. A higher frequency of cigarette smoking and male sex were registered in the CAD than in the diabetic control group. The cases had longer duration of diabetes, higher BMI and lower diastolic blood pressure, a higher total cholesterol level, a higher LDL cholesterol level and a lower HDL cholesterol level than the controls (Table 1).

The genotype distributions of the –429 T/C and –374 T/A gene polymorphisms of the RAGE in cases and controls were compatible with Hardy-Weinberg expectations. There were no differences either in the frequencies of the genotypes of the –429 T/C gene polymorphism or in the frequencies of the genotypes of the –374 T/A gene polymorphism of the RAGE gene between the patients with CAD and those without it (Table 2). Subgroup analyses in males and females also failed to demonstrate any significant differences either in the frequencies of the genotypes of the –429 T/C gene polymorphism or in the frequencies of the genotypes of the –374 T/A gene polymorphism of the RAGE gene between the patients with CAD and those without it (data not shown). Moreover, there were no statistically significant differences either in the frequencies of the alleles of the –429 T/C gene polymorphism (cases: allele C 18.4 %, allele T 81.6 %, controls: allele C 14.7 %, allele T 85.3 %) and/or in the frequencies of the alleles of the –374 T/A gene polymorphism (cases: allele A 31.8 %, allele T 68.2 %,

controls: allele A 32.8 %, allele T 67.2 %) of the RAGE gene between the patients with CAD and those without it.

Multiple logistic regression analysis showed that the duration of diabetes was the only independent risk factor for CAD

TABLE 1
CLINICAL CHARACTERISTICS OF TYPE 2 DIABETIC PATIENTS

Characteristics	Subjects with CAD number (%)	Subjects without CAD number (%)	p
Number	168	241	
Age (years)	59.3 ± 11.4	66.9 ± 9.6	<0.001
Male sex (%)	109 (64.90)	104 (43.2)	<0.001
Duration of diabetes (years)	21.8 ± 7.4	17.8 ± 8.4	0.002
Insulin therapy	114 (67.8)	140 (58.1)	0.4
Age of onset of diabetes	49.6 ± 10.2	49.2 ± 10.9	0.8
HbA_{1c} (%)	8.4 ± 1.6	8.0 ± 1.7	0.2
Systolic blood pressure (mm Hg)	146 ± 20	145 ± 24	0.5
Diastolic blood pressure (mm Hg)	83 ± 10	85 ± 10	0.02
BMI (kg/m^2)	28.7 ± 3.7	27.8 ± 4.5	0.05
History of hypertension (%)	115 (68.5)	162 (67.2)	0.8
Smokers (%)	74 (44.0)	34 (14.1)	<0.001
Total cholesterol (mmol/l)	5.8 ± 1.5	5.5 ± 1.3	0.03
HDL cholesterol (mmol/l)	1.1 ± 0.3	1.2 ± 0.4	0.04
LDL cholesterol (mmol/l)	3.7 ± 1.4	3.2 ± 1.0	0.001
Triglycerides (mmol/l)	2.4 ± 1.3	2.5 ± 1.7	0.5

TABLE 2
GENOTYPE DISTRIBUTIONS OF THE –374 T/A (p VALUE = 0.4, CHI SQUARE = 1.8) AND THE –429 T/C A (p VALUE = 0.3, CHI SQUARE = 2.1) GENE POLYMORPHISM OF THE RAGE GENE IN PATIENTS WITH CAD AND IN DIABETIC CONTROL SUBJECTS

RAGE gene polymorphism	CAD number (%)	Controls number (%)	OR (95 % CI)[1]	p value
		–374 T/A		
AA genotype	13 (7.7)	27 (11.2)	0.7 (0.3–1.3)[2]	0.3[2]
TA genotype	81 (48.2)	104 (43.2)	1.1 (0.7–1.6)[3]	0.7[3]
TT genotype	74 (44.1)	110 (45.6)		
		–429 T/C		
CC genotype	5 (3.0)	4 (1.7)	1.8 (0.5–6.9)[4]	0.4[4]
TC genotype	52 (31.0)	63 (26.1)	1.3 (0.9–2.0)[5]	0.2[5]
TT genotype	111 (66.0)	174 (72.2)		

[1]Odds ratio (95% confidence interval)
[2]Odds ratio (95% confidence interval) and P-value for recessive model (AA vs. TA plus TT)
[3]Odds ratio (95% confidence interval) and P-value for dominant model (AA plus TA vs. TT)
[4]Odds ratio (95% confidence interval) and P-value for recessive model (CC vs. TC plus TT)
[5]Odds ratio (95% confidence interval) and P-value for dominant model (CC plus TC vs. TT).

in type 2 diabetes (OR = 0.95 95% CI = 0.90–1.0; P = 0.04), whereas –429 T/C and –374 T/A gene polymorphisms as well as other variables in the logistic model were not.

Discussion

Our study failed to demonstrate an association between either the –429 T/C or the –374 T/A gene polymorphism of the RAGE gene and CAD in the Slovene population with type 2 diabetes of duration longer than 10 years.

The cardiovascular complications of diabetes represent the leading cause of morbidity and mortality in affected subjects. Beside environmental factors, genetic factors including the gene polymorphism of the RAGE are implicated in the pathogenesis of macrovascular and microvascular complications of diabetes[12,19,20]. Hudson with collaborators demonstrated an effect of the –429 T/C or the –374 T/A gene polymorphism on gene expression, which indicated an influence on RAGE levels[19]. A significant association between the –429 T/C gene polymorphism of the RAGE and diabetic retinopathy was reported in a group of Caucasians with type 2 diabetes, but this association was not confirmed in another group of Caucasians with type 2 diabetes[19,23].

Pettersson-Fernholm with collaborators examined the association between the two functional polymorphisms in the promoter of the RAGE gene (–429 T/C and –374 T/A) and CAD in 996 Finnish patients with type 1 diabetes[20]. In patients with the –374 AA genotype they observed less CAD, and fewer acute myocardial infarctions and peripheral vascular diseases than in those with the TT + TA genotypes. In our study, however, we failed to demonstrate an association between either the –374 T/A or –429 T/C gene polymorphism of the RAGE gene and CAD in 409 Slovenes with type 2 diabetes lasting longer than 10 years. We presume that the lack of relationship between these polymorphisms and CAD in our study may be due to multifactorial nature of the disease, population bias, or sample size. These polymorphisms may make either little or no detectable contribution to CAD. Another explanation for the negative result of an association study might be a type 2 statistical error (i.e., failing to reject the null hypothesis – that there is no difference in genotype distribution between the two groups when it is false). One way to circumvent this problem is to increase the study sample. Moreover, other reasons for differences between our study and the Finish study reported by Pettersson-Fernholm might be that Finish subjects with type 1 diabetes had longer duration of diabetes (more than 20 years) in comparison with our study (more than 10 years); besides, they were much younger than the subjects in our study[20].

In conclusion, we failed to demonstrate an association between either the –429 T/C or the –374 T/A gene polymorphism of the receptor of advanced glycation end products gene and CAD in the Slovene population with type 2 diabetes of duration longer than 10 years.

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GENETSKI POLIMORFIZMI –429 T/C I –374 T/A RECEPTORA ZA PRODUKT KRAJNJE GLIKOZILACIJE (RAGE) NISU ČIMBENICI RIZIKA ZA KORONARNU BOLEST SRCA KOD SLOVENSKE POPULACIJE S DIABETESOM TIPA 2

S A Ž E T A K

Receptor za produkt napredne glikozilacije (RAGE) ima ulogu u stvaranju ateroskleroze kod dijabetičara. Postoje dva polimorfizma u promotoru RAGE gena (–429T/C i –374T/A). Cilj ovog rada bio je pronaći vezu između–429T/C i –374T/A polimorfizma i razvoja koronarne bolesti srca (KBS) u populaciji bolesnika s diabetesom tip 2 u Sloveniji. 168 bolesnika s diabetesom i koronarnom bolesti je uspoređeno s 241 bolesnikom s diabetesom ali bez koronarne bolesti srca. –429T/C i –374T/A RAGE distribucije genotipa kod bolesnika s KBS (–429T/C: CC: 3%, TC: 31%, TT: 66.0%; –374T/A: AA: 7.7%, TA: 48.2%, TT: 44.1%) nisu se značajno razlikovale od onih kod bolesnika bez KBS (–429 T/C: CC: 1.7%, TC: 26.1%, TT: 72.2%; –374T/A: AA: 11.2%, TA: 43.2%, TT: 45.6%). Ova studija nije pokazala značajnu vezu između–429T/C ili –374T/A polimorfizma RAGE gena i koronarne bolesti srca među bolesnicima s trajanjem diabetesa tip 2 većim od 10 godina u Sloveniji.