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Deletion/Deletion Genotype of Angiotensin-I Converting Enzyme Gene is not Associated with Coronary Artery Disease in Caucasians with Type 2 Diabetes

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A B S T R A C T

In this study we analyzed the contribution of genetic variability of the insertion/deletion (I/D) polymorphism of the angiotensin-I converting enzyme (ACE) gene to the predisposition for coronary artery disease (CAD) in a group of patients with type 2 diabetes. The I/D ACE gene polymorphism was tested in 366 Caucasians with type 2 diabetes: 148 cases with CAD and 218 subjects with no history of CAD. We failed to demonstrate that the ACE DD genotype was a risk factor for CAD in Caucasians with type 2 diabetes (OR 2.0, 95 % CI 0.9–4.7; p=0.1). In conclusion, we provide evidence that the ACE deletion/deletion genotype is not a risk factor for CAD in Caucasians with type 2 diabetes.

Keywords: *angiotensin-I converting enzyme, coronary artery disease, diabetes type 2*

Introduction

The prevalence of type 2 diabetes has been rising worldwide due to increasing obesity and decreasing physical activity. In people with type 2 diabetes the leading cause of death is coronary artery disease (CAD)[1]. It is generally accepted that environmental and genetical factors affect the pathogenesis of CAD[2,3]. In Slovenia several candidate genes for cardiovascular disorders have been tested in the general population as well as in diabetic patients[3–8]. Experimental studies and clinical trials show that the renin angiotensin system affects the pathogenesis of CAD and the prognosis of myocardial infarction (MI)[9,10].

The angiotensin-1 converting enzyme (ACE) deletion/deletion (DD) genotype is associated with increased serum concentrations of ACE, which results in enhanced conversion of angiotensin I to II and degradation of bradykinin[11]. In 1992 Cambien et al.[12] showed that the DD genotype of the ACE gene polymorphism is a potent risk factor for MI. Many reports about the role of DD genotype in pathogenesis of CAD and MI have been published since, but only few have so far been performed on patients with type 2 diabetes[13–19]. In previous studies on Slovene population the DD genotype was found to be an independent risk factor for premature MI in general population[20,21].

The aim of this study was to determine whether the ACE DD genotype is a risk factor for CAD in a group of Caucasians with type 2 diabetes.

Materials and Methods

The study population of this cross-sectional analysis consisted of 366 diabetic subjects with type 2 diabetes duration of more than 10 years. In CAD group (148 cases) there were 110 cases with a history of MI (the diagnosis of MI was made according to the criteria of World Health Organization) and 38 cases with angina pectoris confirmed with either abnormal stress testing or abnormal perfusion scan. The control group consisted of 218 diabetics with no history of CAD, no signs of

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ischemic changes on electrocardiogram and no ischemic changes during submaximal stress testing. 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 they had normal electrocardiogram. All the subjects enrolled in the study were Slovenes. The research protocol was approved by the National Medical Ethics Committee. After informed consent was obtained from the patients and control subjects, a detailed interview was made. The physician interviewed each patient 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 defined as 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 confirmed diagnosis of arterial hypertension. Patients were classified as having type 2 diabetes according to current ADA 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.

I/D polymorphism of ACE gene was evaluated as described previously[20]. Genotyping was performed by two researchers (MG, AMŽ), blinded to the CAD status of the patients.

Differences in mean values were analyzed by Student t-test. Chi-square test was used to compare discrete variables. Statistical analysis was performed using the SPSS program for Windows 2000 version 11 (SPSS Inc., Illinois). Statistical significance was set at $p<0.05$. Logistic regression analysis was performed to assess the independent role of the ACE-DD genotype and other variables, including sex, smoking, duration of diabetes, LDL cholesterol level, HDL cholesterol level.

Results

The ACE genotype distributions in cases and controls were compatible with Hardy-Weinberg expectations (Table 1; ACE cases: p=0.93, χ^2=0.007; ACE controls: p=0.69, χ^2=0.15). The characteristics of the cases and control subjects are listed in Table 2 (91.5 % of cases were males). A higher incidence of cigarette smoking (45.9 % vs. 14.2 %; p<0.001) was registered in the CAD than in the control group. The cases had a higher LDL cholesterol level (3.6±1.2 mmol/l vs. 3.2±1.0, p = 0.002), higher BMI (28.9±3.7 vs. 28.0±4.6; p=0.04) and longer duration of diabetes (22.2±6.9 vs. 17.5±8.3; p=0.001) than the controls (Table 2). There were no significant differences in the incidence of hypertension, total cholesterol level, HDL cholesterol level and triglycerides level between the cases and control subjects (Table 2).

TABLE 1
DISTRIBUTION OF ACE I/D GENOTYPES AMONG CAD PATIENTS AND CONTROLS (p=0.3, χ^2=2.1)

Variable	CAD patients (%)	Controls (%)
DD genotype	49 (32.9%)	60 (27.5%)[1]
ID genotype	72 (48.3%)	104 (47.7%)
II genotype	28 (18.8%)	54 (24.8%)

[1] Odds ratio (DD genotype vs. ID genotype + II genotype) = 1.3; 95% CI=0.8–2.0; p=0.3)

TABLE 2
CHARACTERISTICS OF PATIENTS WITH CAD AND CONTROLS

Characteristics	CAD group (%)	Control group (%)	p Value
Number	148	218	
Age (years)	58.6±11.3	65.4±9.9	<0.001
Male sex	97 (65.1)	99 (45.4)	<0.001
BMI (kg/m^2)	28.9±3.7	28.0±4.6	0.04
Arterial hypertension	101 (67.8)	141 (64.7)	0.5
Smoking habit	68 (45.6)	31 (14.2)	<0.001
Diabetes duration (years)	22.2±6.9	17.5±8.3	<0.001
Total cholesterol (mmol/l)	5.7±1.3	5.5±1.4	0.1
HDL cholesterol (mmol/l)	1.1±0.3	1.2±0.3	0.07
LDL cholesterol (mmol/l)	3.6±1.2	3.2±1.0	0.002
Triglycerides (mmol/l)	2.3±1.3	2.6±1.8	0.2

Values are means ± SD

Univariate analysis (Table 2) demonstrated smoking and male sex to be associated with a 4.9-fold (95% CI=2.9–8.2; p<0.001) and a 2.3-fold risk of CAD (95% CI=1.5–3.6; p<0.001), respectively.

TABLE 3
ADJUSTED ODDS RATIOS FOR CARDIOVASCULAR RISK FACTORS IN CAD PATIENTS

Risk factors	OR (95% CI)[1]	p
Diabetes duration	0.95 (0.9–1.0)	0.07
DD genotype[2]	2.0 (0.9–4.7)	0.1
Smoking	1.1 (0.4–3.5)	0.8
Male sex	1.1 (0.4–2.6)	0.9
HDL cholesterol	1.2 (0.4–4.0)	0.8
LDL cholesterol	1.4 (0.9–2.2)	0.2

[1] Odds ratio (95% confidence interval)
[2]ACE insertion/deletion gene polymorphism

Multiple logistic regression analysis of the data (Table 3) showed that the ACE DD genotype was not an independent risk factor for MI in type 2 diabetes (OR=1.4; 95% CI=0.8–2.3; p=0.23).

Discussion

In the study we failed to demonstrate that the ACE DD genotype was a risk factor for CAD in Caucasians with type 2 diabetes.

In a mixed population (196 males+170 females) of 366 patients with type 2 diabetes we failed to demonstrate an association between the ACE DD genotype and an increased risk of CAD (OR=1.3; 95% CI=0.8–2.0; p=0.3). A separate analysis by sex showed an association between the ACE DD genotype and CAD in females (OR=2.6; 95% CI=1.3–5.2; p=0.007), but not in males (OR=0.8; 95% CI=0.4–1.4; p=0.4). In previous study the ACE DD genotype was an independent risk factor for premature MI in the general population (OR= 2; 95% CI 1.1–3.7; p=0.01)[20].

Only few studies have been performed so far on patients with type 2 diabetes, however, the results were inconsistent[13–16]. Several studies including meta-analysis published by Samani et al.[17] demonstrated an association between the ACE DD genotype and MI and CHD. However, Agerholm-Larsen et al.[11] have recently reported a difference in the effect of the ACE DD genotype on CAD, MI, or ischemic cerebrovascular disease between small and large studies: risk of MI and CAD was increased by 47 % and 29 %, respectively, for DD versus ID and II genotypes in small studies but not in large studies[11]. Case control studies may suffer from several biases, which may lead to false positive and false negative results[23,24]. In association studies the survival bias cannot be avoided. It is possible that early mortality in CAD patients with type 2 diabetes is due to the ACE gene and that this could lead to an underestimation of the role of the DD genotype in patients with CAD in our study. Furthermore, a higher percentage of women in our study compared to the study of Ruiz et al.[16] might also be of importance, since CAD mortality associated with diabetes is greater for women than for men[16,25–27].

In our study longer duration of diabetes was demonstrated in the CAD group compared to the control group. Longer duration of diabetes was demonstrated to be an important predictor of total mortality and the mortality due to CAD among women and men with type 2 diabetes[28,29].

In conclusion, the ACE DD genotype is not a risk factor for CAD in Caucasians with type 2 diabetes.

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REFERENCES

1. GRUNDY, S. M., I. J. BENJAMIN, G. L. BURKE, Circulation, 100 (1999) 1134. — 2. LETONJA, M., D. PETROVIČ, Pacing Clin. Electrophysiol., 26 (2003) 2192. — 3. PETROVIČ, D., B. PETERLIN, Cardiology, 99 (2003) 163. — 4. GLOBOCNIK PETROVIC, M., M. HAWLINA, B. PETERLIN, D. PETROVIC, J. Hum. Genet., 48 (2003) 457. — 5. GLOBOCNIK PETROVIC, M., M. HAWLINA, B. PETERLIN, D. PETROVIC, Ophthalmologica, 217 (2003) 219. — 6. PETROVIC GLOBOČNIK, M., K. STEBLOVNIK, B. PETERLIN, D. PETROVIC, Klin. Monatsbl. Augenheilkd., 220 (2003) 873. — 7. PETERLIN, B., M. GLOBOCNIK PETROVIC, J. MAKUC, M. HAWLINA, D. PETROVIC, J. Hum. Genet., 48 (2003) 646. — 8. PETROVIC, D., M. GLOBOCNIK PETROVIC, B. PETERLIN, Cardiology, 100 (2003) 157. — 9. PFEFFER, M. A., E. BRAUNWALD, L. A. MOYE, L. BASTA, E. J. BROWN, T. E. CUDDY, B. R. DAVIS, E. M. GELTMAN, S. GOLDMAN, G. C. FLAKER, N. Engl. J. Med., 327 (1992) 669. — 10. SCHUH, J. R., D. J. BLEHM, G. E. FRIEDLICH, E. G. MCMAHON, E. H. BLAINE, J. Clin. Invest., 91 (1993) 1453. — 11. AGERHOLM-LARSEN, B., B. G. NORDESTGAARD, A. TYBJAERG-HANSEN, Arterioscler. Thromb. Vasc. Biol., 20 (2000) 484. — 12. CAMBIEN, F., O. POIRIER, L. LACERF, Nature, 359 (1992) 641. — 13. ARAZ, M., S. AYNACIOGLU, V. OKAN, I. AKDEMIR, S. AKTARAN, Acta. Cardiol., 57 (2002) 265. — 14. FUJISAWA, T., H. IKEGAMI, G. Q. SHEN, E. YAMATO, K. TAKEKAWA, Y. NAKAGAWA, Y. HAMADA, H. UEDA, H. RAKUGI, J. HIGAKI, Diabetes Care, 18 (1995) 983. — 15. KEAVNEY, B. D., C. R. DUDLEY, I. M. STRATTON, R. R. HOLMAN, D. R. MATTHEWS, P. J. RATCLIFFE, R. C. TURNER, Diabetologia, 38 (1995) 948. — 16. RUIZ, J., H. BLANCHE, N. COHEN, Proc. Natl. Acad. Sci. USA, 91 (1994) 3662. — 17. SAMANI, N. J., J. R. THOMPSON, L. O'TOOLE, K. CHANCER, K. L. WOODS, Circulation, 94 (1996) 708. — 18. TARNOW, L., F. CAMBIEN, P. ROSSING, F. S. NIELSEN, B. V. HANSEN, L. LECERF, O. POIRIER, S. DANILOV, S. BOELSKIFTE, K. BORCH-JOHNSEN, Diabetologia, 38 (1995) 798. — 19. WONG, T. Y., C. C. SZETO, K. M. CHOW, J. C. CHAN, P. K. LI, Am. J. Kidney Dis., 38 (2001) 9. — 20. PETROVIC, D., M. ZORC, V. KANIC, B. PETERLIN, Angiology, 52 (2001) 247. — 21. PETROVIČ, D., I. KEBER, M. ZORC, B. PETERLIN, Pflugers Arch., 431 Suppl. (1996) 197. — 22. 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. — 23. COX, N. J., G. I. BELL, Diabetes, 38 (1989) 947. — 24. PETROVIC, D., M. BIDOVEC, B. PETERLIN, Folia. Biol. (Krakow), 50 (2002) 53. — 25. BARRETT-CONNOR, E. L., B. A. COHN, D. L. WINGARD, S. L. EDELSTEIN, JAMA, 265 (1991) 627. — 26. ROSENGREN, A., L. WELIN, A. TSIPOGIANNI, L. WILHELMSEN, BMJ, 299 (1989) 1127. — 27. PETROVIC, D., M. ZORC, B. PETERLIN, Folia. Biol. (Praha), 46 (2000) 181. — 28. CHO, E., E. B. RIMM, M. J. STAMPFER, W. C. WILLETT, F. B. HU, J. Am. Coll. Cardiol., 40 (2002) 954. — 29. KUUSISTO, J., L. MYKKANEN, K. PYORALA, M. LAAKSO, Diabetes, 43 (1994) 960.

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DELECIJA/DELECIJA GENOTIP ZA ANGIOTENZIN I KONVERTAZU NIJE POVEZAN S KORONARNOM BOLESTI SRCA KOD BIJELACA S DIJABETESOM TIPA 2

S A Ž E T A K

U ovoj studiji analiziran je doprinos genetske varijabilnosti insercije/delecije polimorfizma gena za angiotenzin-I konvertazu na predispoziciju za koronarnu bolest srca u grupi pacijenata s dijabetesom tipa 2. Varijabilnost insercije/delecije gena za angiotenzin-I konvertazu je testirana na 366 bijelaca s dijabetesom tipa 2: 148 ih je imalo koronarnu bolest srca, dok ih 218 nije imalo koronarnu bolest srca. Nije dokazano da je delecija/delecija genotip za angiotenzin-I konvertazu rizični faktor za koronarnu bolest srca kod bijelaca s dijabetesom tipa 2 (OR 2.0, 95% CI 0,9–4,7; p=0,1). Kao zaključak, pružamo dokaz da delecija/delecija genotip za angiotenzin-I konvertazu nije rizični faktor za koronarnu bolest srca kod bijelaca s dijabetesom tipa 2.