

ORIGINAL PAPER**IS THERE AN ASSOCIATION BETWEEN THE ANGIOTENSIN CONVERTING ENZYME GENE POLYMORPHISM AND CAROTID ATHEROSCLEROSIS IN NON-INSULIN-DEPENDENT DIABETICS?****Marija ŠANTL LETONJA¹****Mitja LETONJA^{2,3}****Jovana NIKOLAJEVIĆ-STARČEVIĆ⁴****Dražen POPOVIĆ¹****Danijel PETROVIĆ^{1,4}**¹General Hospital Rakičan, Murska Sobota²General Hospital Ptuj, Ptuj³Medical Faculty, University of Maribor, Maribor⁴Institute of Histology and Embriology, Medical Faculty, University of Ljubljana, Ljubljana

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ABSTRACT

Many studies have investigated the association between the angiotensin-converting enzyme (ACE) gene insertion/deletion (I/D) polymorphism and carotid intima-media thickness (ITM). However, only a few reports so far have studied carotid artery disease by plaque score in non-insulin-dependent diabetes mellitus (NIDDM) patients.

To investigate the impact of genetic polymorphisms of the ACE on carotid atherosclerosis in the Slovenian population with NIDDM, we searched for the association between the ACE I/D gene polymorphism and either ITM or plaque score in subjects with NIDDM.

Study participants were 292 NIDDM patients, randomly selected from one centre, with diabetes duration ≥ 10 years. The ITM and plaque score of the carotid arteries was determined by bilateral B-mode ultrasonography. Polymerase chain reaction was used to evaluate the ACE I/D polymorphism.

The frequency of the allele D was 55.3 %, and the frequency of the allele I was 44.7 %. The mean ITM was 1.08, 1.09 and 1.07 in the ACE DD, ID, and II genotypes, respectively. The ITM and the prevalence of focal plaque assessed by plaque score were not significantly different among the three genotypes in NIDDM patients.

We may conclude that the ACE I/D gene polymorphism is not associated with ITM and plaque score. Therefore it could not be used as a genetic marker of carotid atherosclerosis in NIDDM patients.

Key words: *angiotensin-converting enzyme gene insertion/deletion polymorphism, non-insulin-dependent diabetes mellitus, atherosclerosis; carotid arteries*

INTRODUCTION

Angiotensin-converting enzyme (ACE) is a key enzyme in the renin-angiotensin system, which catalyzes the conversion of angiotensin I to angiotensin II, and the breakdown of bradykinin to kinin. Angiotensin II and bradykinin are peptide hormones with an important role in vascular homeostasis, where they have opposite the effect on vascular tone, smooth muscle cell proliferation, and extracellular matrix production.^{1,2} Thus, chronic exposure to high levels of circulating and tissue ACE may well predispose to vascular wall thickening and atherosclerosis.³

The insertion/deletion (I/D) polymorphism of human ACE gene explains 40-50% of the variance in plasma ACE activity. It has been shown that higher serum ACE activity is present in subjects with the allele D compared to subjects with the allele I.⁴ It is well known that the DD genotype of the ACE gene is an independent risk factor for coronary heart disease in non-diabetic subjects as well as in non-insulin-dependent diabetes mellitus (NIDDM) patients.^{5,6}

The association between ACE I/D polymorphism and increased carotid intimal-medial wall thickness (ITM) has also been examined in several studies with heterogeneous findings, but relatively little is known re-

Table 1. Clinical characteristics of NIDDM patients depending on ACE I/D gene polymorphism

	ACE genotype		
	II	ID	DD
No. of patients	63	135	94
Age y	62.8 ± 8.5	63.6 ± 10	61.6 ± 9.7
Duration of diabetes, y	10 ± 7.9	9 ± 7.7	10.2 ± 9.2
Body weight, kg	87.3 ± 17	87.6 ± 15.3	88.5 ± 15
Body height, cm	167.5 ± 9.2	166.8 ± 14.6	168 ± 9
Waist circumference, cm	108 ± 14.5	108.8 ± 14	108 ± 10.3
BMI ¹	31.1 ± 5.8	32.2 ± 11	31.2 ± 4.2
Duration of hypertension	11.6 ± 8.1	11.2 ± 9.4	12.9 ± 9
Systolic blood pressure, mmHg	136.9 ± 14.9	143.1 ± 19.2	146.7 ± 21.2
Diastolic blood pressure, mmHg	85.9 ± 19.6	83.8 ± 11.5	85.9 ± 11.3
Total cholesterol	5.8 ± 1.5	6.0 ± 1.4	6.1 ± 1.5
LDL cholesterol ²	3.6 ± 0.1	3.8 ± 1.3	3.9 ± 1.4
HDL cholesterol ³	1.1 ± 0.4	1.2 ± 0.5	1.3 ± 0.5
Triglycerides	2.4 ± 1.5	2.3 ± 1.6	2.3 ± 1.6
Smoking %	31.2	46.5	22.3
Carotid ITM ⁴ , mm	1.07 ± 0.14	1.09 ± 0.15	1.08 ± 0.15
Plaque score 0, number	7	16	11
Plaque score 1,2,3, number	37	59	51
Plaque score 4,5,6 number	19	60	32

¹body mass index; ²Low-density lipoprotein cholesterol; ³high-density lipoprotein cholesterol; ⁴Intima-media thickness

garding the association between advanced carotid artery disease assessed with plaque score and ACE I/D polymorphism.⁷⁻¹⁶

To investigate the impact of genetic polymorphisms of the ACE on carotid atherosclerosis in the Slovenian population with type 2 diabetes, we searched for the association between the ACE I/D gene polymorphism and either ITM or plaque score in subjects with NIDDM.

PATIENTS AND METHODS

During a 9-month period NIDDM subjects from the diabetic ambulance of Murska Sobota were enrolled in the cross-sectional retrospective study and referred to the radiology department of the General Hospital Murska Sobota, where carotid ultrasound was performed. All patients were Caucasians of Slovene origin. Before the study, all the tested persons signed a written informed consent. All participants completed a questionnaire concerning clinical information, particularly smoking habits, diagnosis of diabetes and hypertension, and treatment with antidiabetics, lipid lowering and cardiovascular drugs. Body mass index (BMI) was

calculated as weight (kg) divided by the square height (m²). We measured systolic blood pressure (SBP) and diastolic blood pressure (DBP) in the right upper arm of the patients while they were sitting. Hypertension was defined as SBP ≥ 140 or DBP ≥ 90 or those patients who had been treated for hypertension. For blood biochemical analyses, total cholesterol, triglyceride levels, high-density lipoprotein (HDL) cholesterol level, and fasting blood glucose were measured during the fasting condition. The I/D polymorphism of the ACE gene was evaluated as described previously.¹⁷

Carotid Ultrasound

All carotid ultrasounds were performed by an expert radiologist using a high-resolution B-mode ultrasonic machine with a multifrequency transducer (Toshiba Aplio). The region of common carotid artery (CCA), internal carotid artery (ICA) and external carotid artery (ECA) were scanned. All measurements were made at the time of scanning with the instrument's electronic caliper. The IMT of the CCAs at a point 10 mm proximal to the beginning of the dilatation of the bulb was measured. Plaque was defined as a focal IMT thickening, where the IMT was > 1.2 mm. We examined the area of the CCAs, bifurcations, and ICAs, bilaterally and

summarised the number of plaques. The total plaque score reflected the total number of sites with plaques and target from 0 to 6 as described previously.¹⁸

Statistical analysis

Data are expressed as means $\pm$ standard deviations. Clinical characteristics values among the three genotypes were compared with one-way ANOVA with F test. Chi-square test was used to compare genotype distributions. Statistical analysis was performed using the SPSS program for Windows version 16 (SPSS Inc. Chicago, Illinois).

RESULTS

The ACE genotype was determined in 292 NIDDM patients of Slovene origin. The frequency of the D allele was 55.3 %, whereas the frequency of the I allele was 44.7 %. The genotype frequencies of the ACE gene polymorphism in diabetics were: II (21.6 %), ID (46.2 %), and DD (32.2 %). The ACE genotype distribution (table 1) was compatible with Hardy-Weinberg expectations ($\chi^2=1.126$, $p=0.268$).

No significant differences were found among the three genotypes with respect to age, duration of diabetes, body weight and height, BMI, waist circumference, duration of hypertension, diastolic blood pressure, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, smoking, carotid ITM and plaque score (Table 1). Patients with DD genotype had significantly higher systolic blood pressure than patients with ID or II genotype (DD genotype: 146.7 ± 21.2 ; ID genotype: 143.1 ± 19.2 ; II genotype: 136.9 ± 14.9). Diabetics with higher plaque score had an increased ITM thickness (table 2).

DISCUSSION

Our study demonstrated that the D allele of the ACE gene was not associated with increased carotid artery ITM, and we also did not find any association between the allele D and advanced carotid artery disease defined by plaque score, assessed by ultrasonography in patients with NIDDM.

The plaque score grades obtained from ultrasound from a normal ITM to homodynamic important plaque or multiple plaques, is a method of evaluating the relationship between different manifestation of carotid artery pathology and association with genetic and environmental risk factors. We defined plaques as a localized area of thickening of > 1.2 mm because we believe that plaque should be considered to be quanti-

Table 2. Association between the ITM and plaque score ($p<0.001$)

	Plaque score		
	0	1,2,3	4,5,6
ITM ¹ , mm	0.94 ± 0.19	1.09 ± 0.12	1.13 ± 0.13

¹Intima-media thickness

tatively different from general increases in wall thickness measured as IMT. The ITM corresponds to the intima-media complex, which includes endothelial cells, connective tissue and smooth muscle. The pathological process corresponding to ITM thickening is due to smooth muscle hypertrophy, which differs from plaque formation.

There is a well-known association between pulse pressure, which is the marker of arterial rigidity, and allele D of the ACE gene.¹⁹ Castellano and co-workers¹⁰ demonstrated an association between ACE polymorphism and signs of hypertrophy in the vascular wall, namely the ITM. However, many studies in a general population or a low risk population failed to demonstrate an association between the ACE polymorphism and ITM.^{7-9,11} However, three previously published studies, which reported an association between the ACE DD genotype and carotid ITM, did not find a significant association, except in post-hoc analyses, which eliminated subgroups from the original study population.^{10,12,13} On the other hand, an association between the polymorphism of the ACE gene and ITM was reported in subpopulations, such as NIDDM patients,^{14,15} hypertensive patients,¹⁶ and haemodialysis patients,¹⁷ suggesting risk factor-genotype interaction. The meta-analysis of 23 published reports, studying an association between the DD genotype of the ACE gene and the carotid ITM, demonstrated an association, but the association was stronger in high-risk subpopulations.^{22,23} Contrary to these findings, we found no association between the allele D and ITM in the cohort of Slovene NIDDM patients. The genotype distribution (II, 21.6 %; ID, 46.2 %; DD, 32.2 %) was consistent with other published reports in Slovene NIDDM patients.^{24,25} We suppose that the lack of an association between the I/D ACE gene polymorphism and carotid atherosclerosis in our study may be due to the multifactorial nature of the disease. Besides, the negative result may also be attributable to survival bias and selection bias. The findings of the present study are in accordance with another study in the Slovenian population that failed to demonstrate an association between the DD genotype of the ACE gene polymorphism and stroke.²⁶

Only few studies so far have studied the relationship between the allele D and carotid plaque score, and none has demonstrated an association.^{9,27} The find-

ings of our study are in accordance with the findings of these published reports.^{9,27} We speculate that this may be due to the different effect of angiotensin II on cell growth and vascular tone or endothelial function in the early atherosclerotic development in comparison to advanced atherosclerotic lesions.^{28,29} However, we found an association between the ITM and plaque score. This finding supports the hypothesis that ITM is related to atherosclerosis.

CONCLUSION

In the cohort of Slovenian NIDDM patients we failed to demonstrate an association between the I/D ACE gene polymorphism and ITM. Moreover, we did not find an association between the I/D polymorphism of the ACE gene and advanced carotid artery disease assessed by plaque score.

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