ACTA MEDICA SALINIANA

ISSN 0350-364X

Acta Med Sal 2010; 39(2):57-61
DOI:10.5457/ams.178.10

ORIGINAL PAPER

IS THERE AN ASSOCIATION BETWEEN THE ANGIOTENSIN CONVERTING ENZYME GENE POLYMORPHISM AND CAROTID ATHEROSCLEROSIS IN NON-INSULIN-DEPENDENT DIABETICS?

Marija ŠANTL LETONJA[1]
Mitja LETONJA[2,3]
Jovana NIKOLAJEVIĆ-STARČEVIĆ[4]
Dražen POPOVIĆ[1]
Danijel PETROVIČ[1,4]

[1]General Hospital Rakičan, Murska Sobota
[2]General Hospital Ptuj, Ptuj
[3]Medical Faculty, University of Maribor, Maribor
[4]Institute of Histology and Embriology, Medical Faculty, University of Ljubljana, Ljubljana

Slovenia

Received: 11.10.2010
Accepted: 21.11.2010

Correspondence to:
Assoc. Professor Daniel Petrovič, Institute of Histology and Embriology, Medical Faculty of Ljubljana, Korytkova 2, 1105 Ljubljana, Slovenia.
Phone: +386 1 543 7367
Fax: + 386 1 543 7361
E-mail: daniel.petrovic@mf.uni-lj.si

ABSTRACT

Many studies have investigated the association between the angiotensin-coverting 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 determinated by bilateral B-mode ultrasonography. Polymerase chain reaction was used to evaluated 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-coverting enzyme gene insertion/deletion polymorphism, non-insulin-dependent diabetes mellitus, atherosclerosis; carotid arteries*

INTRODUCTION

Angiotensin-converting enzyme (ACE) is a key enzime 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 ± 01.1	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

[1]body mass index; [2]Low-density lipoprotein cholesterol; [3]high-density lipoprotein cholesterol ; [4]Intima-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 preformed. 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 ± 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 (c2=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

[1]*Intima-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-workers[10] 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.

ACKNOWLEDGEMENT

The authors thank Mrs. Visam Bajt, BA, for revising the English.

REFERENCES

1. Kitakaze M, Node K, Takashima S, Minamino T, Kuzuya T, Hori M. Cellular mechanisms of cardioprotection afforded by inhibitors of angiotensin converting enzyme in ischemic hearts: role of bradykinin and nitric oxide. Hypertens Res. 2000; 23: 253-9. doi:10.1291/hypres.23.253 PMid:10821135
2. Daemen MJ, Lombardi DM, Bosman FT, Schwartz SM. Angiotensin II induces smooth muscle cell proliferation in the normal and injured rat arterial wall. Circ Res 1991; 68: 450-6. PMid:1991349
3. Kato H, Suzuki H, Tajima S, Ogata Y, Tominaga T, Sato A et al. Angiotensin II stimulates collagen synthesis in cultured vascular smooth muscle cells. J Hypertens 1991; 9: 17-22. PMid:1848253
4. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest 1990; 86: 1343-6. doi:10.1172/JCI114844 PMid:1976655 PMCid:296868
5. Cambien F, Poirier O, Lecerf L, Evans A, Cambou JP, Arveiler D et al. Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. Nature 1992; 359: 641-4. doi:10.1038/359641a0 PMid:1328889
6. Ruiz J, Blanché H, Cohen N, Velho G, Cambien F, Cohen D et al. Insertion/deletion polymorphism of the angiotensin-converting enzyme gene is strongly associated with coronary heart disease in non-insulin-dependent diabetes mellitus. Proc Natl Acad Sci U S A. 1994; 91: 3662-5. doi:10.1073/pnas.91.9.3662
7. Girerd X, Hanon O, Mourad JJ, Boutouyrie P, Laurent S, Jeunemaitre X. Lack of association between renin-angiotensin system, gene polymorphisms, and wall thickness of the radial and carotid arteries. Hypertension 1998; 32: 579-83. PMid:9740630
8. Zannad F, Visvikis S, Gueguen R, Sass C, Chapet O, Herbeth B et al. Genetics strongly determines the wall thickness of the left and right carotid arteries. Hum Genet 1998; 103: 183-8. doi:10.1007/s004390050804 PMid:9760203
9. Hung J, McQuillan BM, Nidorf M, Thompson PL, Beilby JP. Angiotensin-converting enzyme gene polymorphism and carotid wall thickening in a community population. Arterioscler Thromb Vasc Biol 1999; 19: 1969-74. PMid:10446079
10. Castellano M, Muiesan ML, Rizzoni D, Beschi M, Pasini G, Cinelli A et al. Angiotensin-converting enzyme I/D polymorphism and arterial wall thickness in a general population. The Vobarno Study. Circulation 1995; 91: 2721-4. PMid:7758176
11. Dessi-Fulgheri P, Catalini R, Sarzani R, Sturbini S, Siragusa N, Guazzarotti F et al. Angiotensin converting enzyme gene polymorphism and carotid atherosclerosis in a low-risk population. J Hypertens 1995; 13: 1593-6. PMid:8903616
12. Pujia A, Motti C, Irace C, Cortese C, Biagiotti L, Mattioli PL et al. Deletion polymorphism in angiotensin converting enzyme gene associated with carotid wall thickening in a healthy male population. Coron Artery Dis 1996; 7: 51-5. PMid:8773433
13. Bonithon-Kopp C, Ducimetière P, Touboul PJ, Fève JM, Billaud E, Courbon D et al. Plasma angiotensin-converting enzyme activity and carotid wall thickening. Circulation 1994; 89: 952-4. PMid:8124834
14. Diamantopoulos EJ, Andreadis E, Kakou M, Vlachonikolis I, Vassilopoulos C, Giannakopoulos N et al. Atherosclerosis of carotid arteries and the ace insertion/deletion polymorphism in subjects with diabetes mellitus type 2. Int Angiol 2002; 21: 63-9. PMid:11941276
15. Hosoi M, Nishizawa Y, Kogawa K, Kawagishi T, Konishi T, Maekawa K et al. Angiotensin-converting enzyme gene polymorphism is associated with carotid arterial wall thickness in non-insulin-dependent diabetic patients. Circulation 1996; 94: 704-7. PMid:8772691
16. Jeng JR. Carotid thickening, cardiac hypertrophy, and angiotensin converting enzyme gene polymorphism in patients with hypertension. Am J Hypertens 2000; 13: 111-9. doi:10.1016/S0895-7061(99)00193-4
17. Nergizoğlu G, Keven K, Gürses MA, Aras O, Ertürk S, Duman N et al. Carotid intima-media thickness and ACE-gene polymorphism in hemodialysis patients. J Nephrol 1999; 12: 261-5.
18. Tatti P, Pahor M, Byington RP, Di Mauro P, Guarisco R, Strollo G et al. Outcome results of the Fosinopril Versus Amlodipine Cardiovascular Events Randomized Trial (FACET) in patients with hypertension and NIDDM. Diabetes Care 1998; 21: 597-603. doi:10.2337/diacare.21.4.597 PMid:9571349
19. D. Petrovič, I. Keber, M. Zorc, B. Peterlin. Deletion/insertion polymorphism in the angiotension - converting enzyme gene as a risk factor in the Slovenian patients with coronary heart disease. Eur. J. Physiol. 1996; 431: R197-198.
20. Lee EJ, Kim HJ, Bae JM, Kim JC, Han HJ, Park CS et al. Relevance of common carotid intima-media thickness and carotid plaque as risk factors for ischemic stroke in patients with type 2 diabetes mellitus. AJNR Am J Neuroradiol 2007; 28: 916-9. PMid:17494669
21. H. W. Hense, H. Schunkert, M. Muscholl, A. Luncher, A. Döring, G. Rieger, J. Mol. Med. 1995, 73, 20.
22. Sayed-Tabatabaei FA, Houwing-Duistermaat JJ, van Duijn CM, Witteman JC. Angiotensin-converting enzyme gene polymorphism and carotid artery wall thickness: a meta-analysis. Stroke 2003; 34: 1634-9. doi:10.1161/01.STR.0000077926.49330.64 PMid:12805498
23. Samani NJ, Thompson JR, O'Toole L, Channer K, Woods KL.

A meta-analysis of the association of the deletion allele of the angiotensin-converting enzyme gene with myocardial infarction. Circulation 1996; 94: 708-12. PMid:8772692
24. Globocnik-Petrovic M, Hawlina M, Peterlin B, Petrovic D. Insertion/deletion plasminogen activator inhibitor 1 and insertion/deletion angiotensin-converting enzyme gene polymorphisms in diabetic retinopathy in type 2 diabetes. Ophthalmologica 2003; 217: 219-24. doi:10.1159/000068975
25. Zorc-Pleskovic R, Teran N, Plesković A, Terzić R, Milutinović A. Deletion/Deletion genotype of angiotensin-I converting enzyme gene is not associated with coronary artery disease in caucasians with type 2 diabetes. Coll Antropol 2005; 29: 149-52. PMid:16117314
26. B Peterlin, T Milanez, J Kobal, K Peterlin-Potisk, D Petrovic, A Grad et al. DD Genotype of the angiotensin - converting enzyme gene and stroke in Slovenian population. Eur. J. Physiol. 2000; 439: R38-39. doi:10.1007/s004240000083
27. Kauma H, Päivänsalo M, Savolainen MJ, Rantala AO, Kiema TR, Lilja M et al. Association between angiotensin converting enzyme gene polymorphism and carotid atherosclerosis. J Hypertens 1996; 14: 1183-7. doi:10.1097/00004872-199610000-00005 PMid:8906516
28. Janko Kersnik J, Kuralt M. Are frequent attenders also heart-sink patients? Acta Medico-Biotehnica 2008; 1: 44-50.
29. Unger F. Health is Wealth –Toward a European Lead Market in Health Care. Acta Medico-Biotehnica 2008; 1: 11-18.