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Deletion Polymorphism of the Angiotensin I-Converting Enzyme Gene in Elderly Patients with Coronary Heart Disease

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A B S T R A C T

Controversy exists as to whether the deletion/deletion (DD) genotype of angiotensin l-converting enzyme (ACE) gene polymorphism is associated with coronary heart disease (CHD). There are only a few studies dealing with this issue in the elderly, also with controversial results. The aim of this study was the assessment of correlation between genetic markers and the risk of CHD in the elderly. The results indicated DD genotype importance for CHD in the elderly as proven by discriminant analysis (χ^2=25,77; df=16; p=0.0620). However, the use of univariate method demonstrated no correlation between DD genotype of ACE gene polymorphism and coronary artery disease. D allele of ACE gene was associated with higher activities of ACE plasma. A weak, but increased risk of MI is associated with high frequency of DD genotype in the elderly. Strong correlation between ACE polymorphism and ACE plasma activities was demonstrated.

Key words: *polymorphism ACE gene, ACE activity, CHD, elderly*

Introduction

Although smoking, hypertension, diabetes mellitus, obesity and dyslipidemia are well known risk factors for CHD, many patients without any of these classical risk factors develop MI[1]. This is particularly the case at an advanced age[2]. It is very well known that the general population is getting older and that cardiovascular mortality and morbidity increase with age. Therefore, it is very important to identify all risk factors, including genetic markers, in order to reduce mortality and morbidity of CHD in the entire population, particularly in the elderly. Several genetic investigations, such as case-control study, affected sib-pair analyses, and rat-cross-examinations have attempted to elucidate the genetic pathogenesis of CHD. An insertion /deletion (ID) polymorphism identified in 1990 by Rigat et al.[3], with the insertion or deletion of a 2-base pair fragment in the 17q23 chromosome, is associated with the ACE plasma and cellular activity. Although the presence of the D allele of ACE gene is associated with increased plasma ACE activities[4], which is a rate-limiting enzyme in the renin angiotensin system, the risk for CHD was not consistent. Some investigators suggested that deletion polymorphism in the gene encoding ACE might have a stronger association with CHD than the ID polymorphism[5]. The DD genotype appears to be an independent risk factor not only for myocardial infarction (MI)[6], but also for left ventricular hypertrophy[7], ischaemic or dilated cardiomyopathy[8], family history of sudden cardiac death[9], ischaemic cerebrovascular disease[10] and chronic heart failure[11,12]. The Framingham study[13] revealed that the homozygotus deletion polymorphism of the angiotensin-converting enzyme gene (ACE DD) was associated with increased risk for the hypertensic white male. A few years later this correlation was also confirmed in the Japanese[14]. However, some other studies did not find any strong correlation between the polymorphism of ACE genotype and MI or CHD[15,16]

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The objective of this study was to test the following hypothesis: if specific ACE genetic polymorphism (DD genotype) is related to cardiovascular mortality, then a decreased frequency of »at risk« alleles and an increased frequency of »protective« allele should be present in the elderly, thus affecting their survival outcome. We also wanted to prove whether there was any correlation between insertion /deletion polymorphism of ACE genotype and ACE activity level.

Methods

Study Population

The study group consisted of all patients from one GP outpatient office, Kalinovica in Zagreb, Croatia, older than 65 years. All 346 patients (230 women and 116 men) were retired and had spent almost their whole life in Zagreb. After a thorough clinical examination of patients and patients' records at the beginning of the study, laboratory analyses and ECGs were done. The study complied with the Helsinki Declaration. All subjects received oral and written information about the study prior to giving written informed consent.

Measurements

Basic clinical variables included age, gender, BMI (body mass index), smoking habits, total cholesterol (TC), triglycerides, HDL-C, LDL-C, apo A I, apo B, apo E, fasting glucose, fibrinogen, uric acid, systolic (SBP) and diastolic blood pressure (DBP).

After >10 minutes of rest, SBP and DBP were measured twice by the same physician. Hypertension was defined as the mean SBP of ≥140 mmHg or the mean DBP of ³90 mmHg. Patients currently under antihypertensive medication were, regardless of SBP and DBP levels, also taken into account.

All blood samples were taken after a 12–14 h overnight fast. Venous blood was drawn into evacuated blood-collection tubes without anticoagulant. After clotting, each specimen was centrifuged at 1200x *g* for 20 min and stored at 4 °C until analysis. Samples were analysed the following day. The serum concentrations of cholesterol and triglycerides were measured by standard enzymatic methods (CHOD-PAP and GPO-PAP, respectively) using commercial kits. The serum concentration of HDL-C was measured after precipitation with dextran sulphate and magnesium chloride, while the serum concentration of LDL-C was estimated using Friedewald's formula. Apoproteins AI, B and E were measured by radial immunodiffusion.

Determination of Genotype

The presence (allele I) or absence (allele D) of the 287 bp Alu repeat in intron 16 of the ACE gene were determined by evaluating the size of DNA fragments after PCR amplification, using the primers and PCR conditions as described by Rigat et al.[3]. ID polymorphism was determined by agarose gel electrophoresis with ethidium bromide staining, and DD genotype was reconfirmed by insertion of allele-specific amplification according to Lindpainter's protocol with minor modification.

Clinical events

Diagnosis of coronary heart disease diagnosis was based on the hospital discharge summary containing either the cardiologist's medical records or pathological Q wave in the ECG. In every patient with suspect ECG and no available data on hospital or any other treatment for CHD or myocardial infarction, a vectocardiogram was performed to confirm the diagnosis. Patients with PTCA or coronary artery by-pass without any myocardial infarction or existing angina pectoris were also considered as CHD patients.

Statistical analysis

The difference in genotypes or allele distribution between the patients with and those without CHD was examined using χ^2 test. The significance level was set at $p<0.05$. The correlation between ACE gene polymorphism and CHD was analysed by discriminant analysis. The statistical analysis was performed with SPSS 9.0 software (SPSS Inc., Chicago, IL, USA, 1999).

Results

Patient characteristics

The analysis of data from 346 subjects showed that 90 (60 females and 30 males) had CHD, while 256 subjects had no signs of CHD. Among those with CHD, 27 had MI (12 inferior, 3 posterior, 3 inferoposterior, 5 anteroseptal, 1 lateral, 3 anterior, 1 anterolateral and 1 high lateral). Thirty-five patients had angina, 19 of them were without previous MI and 16 had angina as a late complication of MI. Twenty-six subjects had pathologic Q wave in ECG, but 6 of them were unaware that they had survived MI. Therefore, a vectocardiogram was performed which proved chronic sequellae of the MI. One patient had PTCA without MI, angina or pathologic Q wave.

Genotype distribution of ACE gene polymorphism

From 346 examinees, for full and qualitative analyses of genome, 322 samples (214 female and 108 male) were accessible.

The distribution of DD, ID and II genotype was 35.4%, 48.1% and 16.4%, respectively. Statistically significant correlation between ACE gene polymorphism and CHD was not demonstrated for the entire group either in males or females analysed separately.

Relative predictive value of quantitatively measured ACE gene polymorphism and other risk factors (fasting sugar, TC, triglycerides, HDL-C LDL-C, uric acid, apo A-l, apo B, apo E, systolic and diastolic blood pressure and cigarette smoking) for CHD was also estimated by discriminant analysis.

TABLE 1
ANGIOENSIN CONVERTING ENZYME (ACE) GENE POLYMORPHISM AND CORONARY HEART DISEASE (CHD) IN THE ELDERLY

ACE Genotype	Gender	CHD (Number of examinees)			Total
		Yes	p*	No	
DD	Total n=322	28	0.18724	86	114
ID		41		114	155
II		20		33	53
DD	Female n=214	17	0.24014	49	66
ID		27		82	109
II		15		24	39
DD	Male n=108	11	0.55799	37	48
ID		14		32	46
II		5		9	14

*p<0.05

TABLE 2
STRUCTURE MATRIX OF DISCRIMINANT FUNCTION

Variable	Discriminant function
Uric acid	–0.67655
Fibrinogen	0.37037
DD genotype	0.34958
Angiotensin converting enzyme (ACE) activity level	–0.28986
Apoprotein E (Apo E)	0.26907

Results indicated DD genotype importance for the development of CHD at an older age. The obtained discriminant function was not statistically significant at the conventional level of significance (χ^2=25.77; df=16; p=0.0620). Since it was significant at 7% level, it might be described as exploratory. Table 2 shows the structure matrix for the obtained discriminant function. Discriminant function was defined by five variables (uric acid, fibrinogen, polymorphism ACE gene (DD genotype), ACE activity level, and Apo E).

Centroid of compared groups on discriminant function is shown in Table 3.

In the observed group classified as diseased (n=90), with determined risk factors for CHD, 60.0% were estimated as diseased, and 40.0% as healthy due to the existence of unknown risk factors by which the »healthy« group was determined within the diseased group. In the other group classified as healthy (n=256), 37.5% were estimated as diseased and 62.5% as healthy because CHD had not yet developed despite the existing risk factors.

TABLE 3
CENTROID OF COMPARED GROUPS ON THE DISCRIMINANT FUNCTION

Under observation	Discriminant function
Diseased	–0.47188
Healthy	0.16590

TABLE 4
RESULTS OF CLASSIFICATION

Under observation	Estimated classification in the group	
	Diseased	Healthy
Diseased	54	36
n=90	60.0%	40.0%
Healthy	96	160
n=256	37.5%	62.5%

On the basis of the Centroid and according to Tale 2 »healthy« differ from »diseased« with higher values of uric acid and higher values of ACE activity, and lower values of fibrinogen, II genotype and lower values of Apo E. Subjects were classified with 61.85% of accuracy (Table 4).

Reverse correlation of DD genotype and ACE activity level related to CHD was revealed by discriminant analysis. The comparison of ACE activity level and DD genotype distributions within genders and in the entire group was performed by univariate analysis (Table 5).

TABLE 5
ANGIOTENSIN CONVERTING ENZYME (ACE) GENE POLYMORPHISM AND ACE ACTIVITY LEVEL IN THE ELDERLY

ACE (U/L)	Gender	ACE Genotype (n)			Total	p*
		DD	ID	II		
8–52	Total	89	132	50	271	
≥ 52	n=322	25	23	3	51	0.02462
8–52	Female	52	91	38	181	
≥ 52	n=214	14	18	1	33	0.03440
8–52	Male	37	41	14	108	
≥ 52	n=108	11	5	2	18	0.28363

*p<0.05

Statistically significant correlation between plasma ACE activity levels and DD genotype was found for the entire group ($p=0.02462$) and also for female ($p=0.03440$) although not for male patients.

Discussion

Although 'classical' risk factors for CHD are well known, many patients without any of these risk factors develop myocardial infarction, particularly at an advanced age. It is very well known that the general population is becoming older and that cardiovascular mortality and morbidity increase with age. Therefore, it is very important to identify all risk factors in order to reduce mortality and morbidity of CHD in the entire population, especially in the elderly.

The effect of the ACE D allele had been discussed within the context of CHD in early 1992, when Cambien et al. first reported an association of ACE DD genotype with MI survivors. Observation of patients at »low« risk according to classical risk factors showed ACE DD genotype as an independent risk factor[5]. At that time Mattu et al. reported an association of the ACE D allele with CHD in low risk patients. However, the association was not present when the BMI was taken into account[17]. Since then, several other groups have investigated the possible relationship of ACE polymorphism with CHD. A recent review that applied meta-analysis in examining the cause-and-effect relationship between ACE ID polymorphism and cardiovascular renal risk could not identify a significant association with hypertension but suggested its role as a marker of atherosclerosis, cardiovascular complications and diabetic nephropathy. Most of them showed that DD genotype was a very strong risk factor for coronary events (MI, fatal MI, and sudden death)[5,6,10,18]. Numerous studies showed how DD genotype was associated with increased risk for hypertension[19–26], and could be a significant risk factor and predictor for the development of malignant hypertension[25,27].

This study was dedicated to individuals of an advanced age. Using the univariate method, statistically significant correlation between ACE D allele and CHD was not determined for the entire group, or when males and females were analysed separately. Distribution of ACE gene polymorphism was in favour of ID genotype. One possible explanation for the low level of DD at this age is that ACE DD is not associated with longevity, and ACE genotype proportion in senior subjects was different from that in young and middle-aged subjects. Therefore, subjects with DD genotype die or suffer MI at an early age. On the other hand, some studies have failed to show any correlation between the DD genotype and CHD. A large prospective American physician's study showed no association between ACE ID polymorphism and the prevalence of MI or ischemic heart disease[28]. Some other studies could not confirm any associations between ACE ID polymorphism and CHD either. Only a few age-related studies have been conducted. Some of them confirm the relation between the renin-angiotensin system and systolic and diastolic blood pressure in the elderly. The difference between the studies may partially be explained by the differences in sensitivity of the methods used to assess association with CHD. Our study shows that, according to qualitative analysis, deletion polymorphism of ACE gene had no influence on the occurrence of CHD in the elderly. However, multifactorial quantitative analysis showed statistical significance. These results suggest that the D allele of the ACE gene is a risk factor for CHD, also in the elderly, but not at the conventional level of significance. The slight significance at this age might be explained as a possibility that DD genotype is not associated with longevity and that the presence of »protective« allele in a qualitative study might be affecting survival.

ACE plays a major role in the cardiovascular system and is involved in the metabolism of two important vasoactive peptides, angiotensin and bradykinin, which are involved in the modulation of vascular tone and proliferation of smooth muscle cells. Subjects with DD genotype appear to have a higher ACE level[17], which is responsible for increased angiotensin II levels and subsequently for vasoconstriction. In the study, we also examined the influence of ACE DD genotype on ACE plasma activities. As many other studies[28], we showed a significant correlation between ACE polymorphism and ACE plasma activities for the entire group and for females when observed separately. Subjects with DD genotype appear to have the highest ACE activity. This is in accordance with the results obtained in the study by Rigat et al.[3]. We also confirmed that the ACE plasma activity was stable and related to ACE polymorphism.

Conclusion

We proved that DD genotype was, however slight, nevertheless a risk factor for CHD in the elderly. Strong correlation between ACE polymorphism and ACE plasma activities was demonstrated. A possible reason for such weak linkage between DD genotype and CHD is that ACE DD is not associated with longevity, or with frequently present asymptomatic disease. A more likely and more reasonable explanation could be that life style and environment change the influence of the genetic marker. We did not examine the influence of other diseases often associated with MI. Other limitations underlying this study were also present. A possible synergistic interaction of the ACE gene polymorphism with other genes, as well as an interaction between ACE ID polymorphism and conventional risk factor, cannot be excluded.

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POLIMORFIZAM ACE GENA U PACIJENATA STARIJE ŽIVOTNE DOBI SA KORONARNOM BOLESTI

SAŽETAK

Povezanost delecijskog alela (DD genotip) angiotenzin konvertirajućeg enzima (ACE) sa koronarnom bolesti srca (KBS) je dvojbena. Malo je studija posvećeno tom problemu za stariju životnu dob, čiji rezultati su također dvojbeni. Cilj ovog rada je bio procijeniti povezanosti genetskih markera i rizika za KBS u starosti. Rezultati pokazuju da DD genotip ima prediktivnu vrijednost za KBS u starosti prikazano diskriminacijskom analizom (χ^2=25,77; df=16; P=0.0620). Međutim upotrebljavajući univarijatnu metodu povezanost DD genotipa i KBS nije dokazana. Vrijednost aktivnosti plazmatskog ACE u direktnoj je povezanosti s delecijskim polimorfizmom ACE gena. Zaključak: Povezanost DD genotipa i rizika za KBS u starijoj životnoj dobi također postoji. Prikazana je jaka povezanost ACE polimorfizma i vrijednosti aktivnosti plazmatskog ACE.