AGE (2012) 34:583–595
DOI 10.1007/s11357-011-9270-0

Angiotensin-converting enzyme deletion allele is beneficial for the longevity of Europeans

Matea Zajc Petranović · Tatjana Škarić-Jurić · Nina Smolej Narančić · Željka Tomas · Petra Krajačić · Jasna Miličić · Maja Barbalić · Spomenka Tomek-Roksandić

Received: 10 February 2011 / Accepted: 9 May 2011 / Published online: 26 May 2011

Abstract The human angiotensin converting enzyme (ACE) gene is one of the most investigated candidate genes for cardiovascular diseases (CVD), but the understanding of its role among the elderly is vague. Therefore, this study focuses at: (a) testing the association of ACE polymorphism with CVD risk factors among the elderly, and (b) detecting the possible unequal distribution of ACE genotypes between senescent and younger segments of the European populations. The association of ACE I/D polymorphism with CVD health status [hypertension (HT), obesity, dislypidemia] in 301 very old subjects (88.2±5 years; F/M=221/80) was tested by means of logistic regression analysis. The meta-analysis of D allele frequency in general vs. elderly (80+ years) groups was conducted using all publicly available data for European populations comprising both age cohorts. Multiple multinomial logistic regression revealed that within this elderly sample, age (younger olds, 80–90 years), female sex (OR=3.13, 95% CI=1.59–6.19), and elevated triglycerides (OR=2.53, 95% CI=1.29–4.95) were positively associated with HT, while ACE polymorphism was not. It was also established that the DD genotype was twice as high in 80+ cohort compared to general population of Croatia ($p<0.00001$). This trend was confirmed by the meta-analysis that showed higher D allele frequencies in olds from nine of ten considered European populations (OR=1.19, 95% CI=1.08–1.31). The data in elderly cohort do not confirm previously reported role of ACE DD genotype to the development of HT. Moreover, meta-analysis indicated that ACE D allele has some selective advantage that contributes to longevity in majority of European populations.

Keywords Aged · Aged 80 and over · Longevity · ACE I/D polymorphism · Hypertension · Meta-analysis

M. Zajc Petranović (✉) · T. Škarić-Jurić · N. Smolej Narančić · Ž. Tomas · P. Krajačić · J. Miličić
Institute for Anthropological Research,
Zagreb, Croatia
e-mail: matea@inantro.hr

M. Barbalić
Human Genetics Center,
University of Texas Health Science Center,
Houston, TX, USA

S. Tomek-Roksandić
Department of Gerontology,
Dr Andrija Štampar Institute of Public Health,
Zagreb, Croatia

Introduction

Cardiovascular diseases (CVDs) are multifactorial diseases involving heart, brain, and peripheral circulation. They are among the leading causes of death and disease burden in both high- and low-income countries (Lopez et al. 2006). According to the information published by Central Bureau of Statistics of the Republic of Croatia, cardiovascular and cerebrovascular diseases were the

most common cause of death in 2009 for both men and women, constituting over 39% of all deaths (Croatian Bureau of Statistics 1992).

The human angiotensin converting enzyme gene (ACE gene) is one of the most investigated candidate genes for CVD. It converts angiotensin I into a physiologically active angiotensin II, which is a potent vasopressor and aldosterone-stimulating peptide that controls blood pressure (BP) and fluid electrolyte balance. ACE also degrades bradykinin to inactive fragments, reducing the serum levels of endogenous vasodilators (Brewster and Perazella 2004; Fleming 2006). The insertion or deletion (I/D) of a 287-bp-long Alu repeat element in the intron 16 of this gene was proven associated with the altered levels of circulating ACE enzyme (Rigat et al. 1990) as well as with cardiovascular pathophysiologies (Cambien et al. 1992; Jeunemaitre et al. 1992a; Keavney et al. 1995). Some of the conducted studies showed positive association of DD genotype and increased risk of myocardial infarction and atherosclerosis (Agerholm-Larsen et al. 2000; Sayed-Tabatabaei et al. 2003). However, a great number of studies, including meta-analyses, associating ACE I/D with hypertension (HT), cardiomyopathy, and coronary arthery disease showed controversial results (Jeunemaitre et al. 1992b, 1997; Lindpaintner et al. 1995; Staessen et al. 1997; Sharma 1998; Dikmen et al. 2006; Xu et al. 2008; Vaisi-Raygani et al. 2010). Furthermore, recent studies have unravelled roles of the renin–angiotensin system (RAS) and, particularly, its main effector molecule angiotensin II in inflammation, autoimmunity, and aging (Papadopoulos et al. 2000; Pueyo et al. 2000; Suzuki et al. 2003; Kitayama et al. 2006; de Cavanagh et al. 2007; Platten et al. 2009; Benigni et al. 2010).

Although widely explored, the contribution of ACE I/D and plenty other genetic and environmental risk factors across age spectrum is still unclear. Given the fact that our sample consists of 80 years old and older subjects in rather good physical and mental condition, it is plausible to expect that they have some protective factors that contributed to their good health and longevity. Therefore, we hypothesize that ACE I/D frequencies differ across population age distribution as a consequence of its association with CVD, its risk factors, and hence mortality.

As HT is recognized as one of the main CVD risk factors (Clarke et al. 2009), in this study, we will first investigate the effect of ACE I/D polymorphism on HT in a Croatian 80+ years population. Furthermore, we will test the association between ACE I/D polymorphism and longevity comparing our sample with previously reported frequencies for the general Croatian population aged 18–80 years (Barbalić et al. 2004). Finally, to test whether I/D allele frequency trend really exists, we will conduct a meta-analysis for both general and 80+ population for the allele frequency distribution in European populations.

Materials and methods

Subjects

A field study was carried out from 2007 to 2009. Participants were recruited from all 11 homes for elderly and infirm in the property of the City of Zagreb, and two private homes located in Zagreb County. The 80 years old and older residents were invited to participate voluntarily, and they all signed written, informed consent. Altogether, 301 examinees participated in this study (222 women, 79 men, age range 80–101 years, mean 88.2±5 years). This sample represents 2.86% of the population of Zagreb City in the age group 80+ years (Croatian Bureau of Statistics 1992).

Study protocol

The study protocol was approved by the Ethics Committee of the Institute for Anthropological Research. It consisted of an extensive interview including the mini-mental state examination, the mini nutritional assessment, BP measurement, short anthropometry, ultrasound measurement of bone mineral density, and collection of venipuncture specimens for biochemical parameters and genetic analyses. Questionnaire included detailed questions regarding sociodemographic status, health status, medical history, nutritional habits, and examinee's satisfaction with his/her quality of life. Trained examiners filled out a questionnaire during a face-to-face interview. Only subset of the obtained data is presented here and explained in more details.

Short anthropometry was undertaken following standard international biological programme protocol (Weiner and Lourie 1981). Body mass index (BMI) was calculated as weight in kilograms divided by squared height in meters (kg/m^2). On the basis of BMI (WHO Classification; WHO 2000), subjects

were divided into three groups: underweight (BMI< 18.50 kg/m^2), normal (BMI 18.50–24.99 kg/m^2), and overweight (BMI≥25 kg/m^2).

BP was measured in a seated position by the physician using a standard mercury sphygmomanometer and stethoscope after participants rested for 15 min. The selection criteria for hypertensive cases were any of the following: systolic BP equal to or higher than 140 mmHg, diastolic BP equal to or higher than 90 mmHg, subject's statement that she/he is hypertensive (indicating previous diagnosis of HT by personal physician), and subject's drug treatment history of HT.

Whole blood samples were obtained by venipuncture and collected for every subject into three tubes for the following analyses: blood serum biochemical analyses, hematological analysis, and DNA extraction. Serum and blood cells analyses were performed following standard internationally agreed procedures. Biochemical analysis included triglyceride and cholesterol measurements [total, high-density and low-density lipoprotein cholesterol (HDL and LDL), and atherosclerosis index].

ACE genotyping

DNA was extracted by a salting-out method (Miller et al. 1988). Genomic DNA was amplified by polymerase chain reaction (PCR) with primers 5′-CTG GAG ACC ACT CCC ATC CTT TCT-3′ (forward) and 5′-GAT GTG GCC ATC ACA TTC GTC AGA T-3′ (reverse). PCR cycling was in a touchdown regime described previously (Kalaydjieva et al. 1999).

The PCR products, 490-bp-long (I) and 190-bp-long (D) fragments, were analyzed by agarose gel electrophoresis using 2%-agarose gel and visualized with SYBR Gold stain.

A selection of studies for meta-analysis

In order to compare ACE I/D polymorphism D allele frequency distribution in Croatians and other Europeans in general and 80+ populations, electronic databases (PubMed, MEDLINE and Science Direct) were searched up to April 2010 for similar studies. The keywords used for the search were: (ACE I/D OR ACE id OR ACE indel OR ACE Alu) AND (longevity OR senescence OR general population). All languages were searched initially, but only English language articles were selected. Furthermore, the references of all selected publications were searched for additional studies. Slovakian 80+ age group was obtained through personal correspondence with Prof. Daniela Sivakova.

The primary search generated more than 647 potentially relevant articles, 38 of which met the inclusion criteria. Studies were selected if they met all of the following:

(a) Studied populations were Europeans.
(b) Studies contained D allele frequency data for general and/or older (80+ years) populations.
(c) There were available data concerning D allele frequency for both age subsets from the same European country.

The studies of ten European populations satisfied the previous conditions: Croatia, Denmark, Finland, France, Germany, Italy, Russian Federation, Slovakia, Spain, and United Kingdom. A list of selected studies is presented in Table 1. All participants in the conducted studies were Caucasians. If there was more than one study conducted in the same country for a certain age group, the reported D allele frequency was calculated by sample size weighting (pondering).

Statistical analysis

We determined ACE ID genotype frequencies by direct counting, and allele frequencies were calculated from the genotype frequencies. The Hardy–Weinberg equilibrium (HWE) was evaluated by the Chi-square (χ^2) test. We tested differences in CVD risk factors between sexes using Fisher's exact test and Pearson's Chi-square test. Same factors were analyzed using t test in hypertensives vs. nonhypertensives.

Several multivariate logistic regression models were tested to verify the combined effect of several risk factors on HT, and the best regression model was taken into account. The following variables were tested: age, sex, BMI, waist/hip ratio, cholesterol (total, HDL, and LDL) and triglyceride serum levels, ACE ID genotypes and I and D alleles frequency. The analyses were performed by SPSS 10.0 statistical package for Windows (SPSS, Chicago, IL, USA), with statistical significance set at $p<0.05$.

Meta-analysis was performed to further investigate the association of ACE D allele with longevity in European populations using Stata 10 (Stata Corporation, College Station, TX, USA). The odds ratio (OR) was

Table 1 Characteristics of 43 studies included in the meta-analysis presented by countries

Country	Reference	Age (years)	Age group	Sample size	Gender distribution M/F	Source of sample	Genotype distribution			Allele frequencies (%)	
							DD	ID	II	D allele	I allele
Croatia	Barbalić et al. 2004	18–80	1	172	Not reported	Healthy general population	38	94	40	0.49	0.51
	Present study	80–101	2	301	79/222	Population from retirement homes in Zagreb	129	110	62	0.61	0.39
Denmark	Agerholm-Larsen et al. 1997	43.1±0.2	1	3,191	1,737/1,454	General population from Copenhagen	Not reported			0.52	0.48
	Bladbjerg et al. 1999	20–64	1	199	124/75	Healthy young blood donors	51	102	46	0.51	0.49
	Hadjadj et al. 2007	44.8±11.0	1	382	Not reported	EURAGEDIC study	Not reported			0.51	0.49
	Bladbjerg et al. 1999	>100	2	185	47/141	Volunteers from centenarian population	49	95	41	0.52	0.49
Finland	Fuentes et al. 2002	35–55	1	454	167/288	FINRISK survey	Not reported			0.57	0.43
	Islam et al. 2006	34.35±3.16	1	224	121/103	Cardiovascular Risk in Young Finns Study	79	106	39	0.59	0.41
	Hadjadj et al. 2007	44.8±11.0	1	468	Not reported	EURAGEDIC study	Not reported			0.56	0.44
	Myllykangas et al. 2000	>85	2	203	40/163	Vantaa 85+ study	65	105	33	0.58	0.42
France	Marre et al. 1997	44±9	1	346	180/166	Healthy nondiabetic general population	117	154	75	0.56	0.44
	Hadjadj et al. 2007	44.8±11.0	1	273	Not reported	EURAGEDIC study	Not reported			0.54	0.46
	Schachter et al. 1994	>99	2	338	44/294	Centenarian population	134	148	56	0.62	0.33
	Blanche et al. 2001	>100	2	560	94/466	Centenarian population	196	261	103	0.58	0.42
	Richard et al. 2001	>80	2	152	Not reported	General population	48	67	37	0.54	0.46
Germany	Schunkert et al. 1994	45–59	1	290	149/141	General population of Augsburg	Not reported			0.54	0.46
	Busjahn et al. 1997	34±14	1	139	34/105	91 monozygotic and 41 dizygotic twins (included only one member of each pair)	33	79	37	0.52	0.48
	Mondry et al. 2005	41.24±12.66	1	719	419/399	General population of Weisswasser	193	356	170	0.52	0.48
	Luft 1999	80+	2	349	Not reported	80+ population of Berlin	118	159	72	0.57	0.43
Italy	Arbustini et al. 1995	35±13	1	290	210/80	Healthy blood donors	120	124	46	0.63	0.37
	Paterna et al. 2000	37.5±9.3	1	201	74/127	Healthy control for migraine patients (no history of CVD)	75	101	25	0.62	0.38
	Di Pasquale et al. 2004	25–55	1	684	443/241	Healthy volunteers (free of CVD)	225	335	124	0.57	0.43
	Paolisso et al. 2001	>100	2	41	15/26	Healthy population	15	20	6	0.61	0.39

	Panza et al. 2003	100±2	2	82	20/62	Healthy population from Southern Italy	38	34	10	0.67	0.33
	Nacmias et al. 2007	102.4±4.6	2	111	23/88	Healthy control for Alzheimer disease patients (no neurological disorder)	57	40	14	0.69	0.31
	Corbo et al. 2008	>77 (82.2±4.8)	2	151	73/78	Healthy subjects in post-reproductive age: LONCILE study (Salerno, sothern Italy)	61	71	19	0.64	0.36
Russia	Dolgikh et al. 2001	25–64	1	945	603/342	WHO Monica (Novosibirsk population)	Not reported			0.52	0.48
	Miloserdova et al. 2001	34.2±2.37	1	50	Not reported	Random sample of Moscow population	Not reported			0.56	0.44
	Nazarov et al. 2001	32±10	1	449	269/180	111 students of St Petersburg University and 338 blood donors (Russians of European and Siberian descent, 3:1)	Not reported			0.50	0.50
	Miloserdova et al. 2001	83.17±3.39	2	50	Not reported	Random sample of Moscow population	Not reported			0.68	0.32
Slovakia	Dankova et al. 2009	40–60 (49.54)	1	167	45/122	Volunteers recruited at random (different localities in Slovakia)	47	82	38	0.53	0.47
	Sivakova et al. 2009	82.85±2.68	2	61	Not reported	Physically and mentally fit volunteers from different regions of Slovakia	20	27	14	0.55	0.45
Spain	Riera-Fortuny et al. 2005	60.2±9.5 (35–79 years)	1	182	127/55	Healthy control coronary heart disease nor CVD risk factors	67	83	21	0.60	0.40
	Hernández Ortega et al. 2002	54±10	1	315	223/92	Randomly selected Gran Canaria Island population with no history of CVD	137	132	136	0.64	0.36
	Alía et al. 2005	44	1	104	46/58	Healthy sarcoidosis controls	34	51	19	0.57	0.43
	Villar et al. 2008	18–75	1	364	182/182	Randomly selected Canarian (Spanish) population from all seven islands	152	155	57	0.63	0.37
	Alvarez et al. 1999	>85	2	117	Not reported	Healthy Alzheimer controls	43	58	16	0.62	0.38
United Kingdom	Samani et al. 1996	20–77	1	537	299/238	Healthy control for CHD from Leicester (N=237) and Sheffield (N=300)	158	259	120	0.54	0.46
	Sharma et al. 1997	41.4±11.9 (19–70)	1	146	59/87	Volunteers attending the Regional Blood Transfusion Service	44	63	39	0.52	0.48
	Garrib et al. 1998	38.2	1	100	Not reported	Healthy control for sarcoidosic patients	Not reported			0.53	0.47
	Jackson et al. 2000	18–65	1	478	Not reported	Blood donors from Cambridge	135	241	102	0.53	0.47
	Galinsky et al. 1997	>79	2	270	100/170	Cambridge City population (longitudinal study of cognitive function and aging)	87	128	55	0.56	0.44
	Kehoe et al. 1999	80.8±4.5	2	111	Not reported	Healthy Alzheimer controls (London population)	41	48	22	0.59	0.41

Age data in this table are not presented uniformly because they were reported differently in the papers: some authors preferred age range, others mean age (SD) or just reported that subjects were over a certain age. We divided them in two groups: group one, for subjects aged 18–80 years, and group two, for those aged 80+ years (or if the mean age of the entire group was 80+). Each study group consisted of both females and males. Genotype distribution is reported in absolute numbers.

Table 2 Basic description of distribution of CVD risk factors among Croatian elderly subjects (80–101 years)

Variable	Gender	*N*	Mean/prevalence	SD	*p* Values
Age (years)	Male	80	88.1	3.4	0.536
	Female	221	88.4	3.7	
Systolic BP (mmHg)	Male	80	138.2	23.8	0.653
	Female	221	132.6	22.8	
Diastolic BP (mmHg)	Male	80	73.7	11.7	0.431
	Female	221	70.0	11.6	
BMI (kg/m^2)	Male	80	27.6	4.8	0.450
	Female	221	27.5	4.4	
Total cholesterol (>5.0 mmol/l)	Male	33	41.2%		*0.000*
	Female	161	72.8%		
HDL cholesterol (F<1.20; M<1.0)	Male	17	21.2%		0.298
	Female	55	24.8%		
LDL cholesterol (>3.0 mmol/l)	Male	35	43.7%		*0.000*
	Female	150	67.8%		
Triglycerides (>1.70 mmol/l)	Male	18	22.5%		*0.001*
	Female	97	43.8%		

Differences between sexes were tested using Pearson's χ^2 test and Fisher's exact test for qualitative variables (cut-off values are listed in parenthesis)

used to compare contrasts of alleles between general population and senescents. Data were combined using random effects method (Mantel-Haenszel) and fixed effects method (Der Simonian and Laird). The between-population heterogeneity was evaluated using the χ^2-based Cochran's Q statistic (Petiti 1994) and the inconsistency index (I^2; Higgins et al. 2003). Publication bias or other small study related bias was evaluated using the rank correlation method of Begg and Mazumdar and fixed effects regression method of Egger et al. (1997).

To identify potential influential studies (countries), we calculated the effects estimates (ORs) by removing an individual study each time and then checked if the overall significance of the estimate or of the heterogeneity statistics was altered. Due to the composite nature of each country's sample, cumulative and meta-regression analysis could not be assesed.

Results

The examined CVD risk factors are shown in Table 2. Women had significantly elevated total cholesterol ($p<0.000$), LDL cholesterol ($p<0.000$), and triglyceride level ($p<0.001$) compared to men. The majority of the subjects were hypertensive, although the average BP was normal (below 140/90 mmHG), indicating adequate medical treatment. In Table 3, we compared the distribution of CVD risk factors in normotensive and hypertensive subjects. Hypertensives were more likely to be younger (87.55 vs. 88.65 years) and to have elevated HDL cholesterol ($p<0.03$).

Of the investigated risk factors, multivariate logistic regression showed that age (younger olds, 80–90 years), female sex (OR=3.13: 95% CI=1.59–6.19), and elevated triglyceride concentration (OR=2,53: 95% CI=1.29–4.95) had significant influence on incidence of HT, while ACE genotype, BMI, waist/hip ratio, and cholesterol concentration did not (Table 4).

Table 3 Differences in CVD risk factors mean values in normotensives (NT) and hypertensives (HT) tested using independent samples *t* test

Variable	HT status	*N*	Mean	SD	*p*
Age (years)	NT	51	88.65	4.10	*0.042*
	HT	250	87.55	3.35	
BMI (kg/m^2)	NT	45	27.38	4.24	0.321
	HT	234	28.11	4.61	
Total cholesterol (mmol/l)	NT	51	5.87	4.06	0.522
	HT	248	5.50	1.13	
HDL cholesterol (mmol/l)	NT	51	1.28	0.35	*0.039*
	HT	248	1.39	0.35	
LDL cholesterol (mmol/l)	NT	50	3.23	0.84	0.290
	HT	248	3.38	0.96	
Triglycerides (mmol/l)	NT	51	1.83	1.61	0.146
	HT	248	1.60	0.86	

Table 4 CVD risk factors in multivariate logistic analysis for the hypertension

		OR	95% CI	*p* Value
ACE I/D	ID versus II	1.04	0.46–2.42	
	DD versus II	1.04	0.44–2.45	
Age (younger olds are referent)		0.90	0.82–0.98	*
Sex (males are referent)		3.13	1.59–6.19	**
Triglycerides (<1.70 mmol/l is referent)		2.53	1.29–4.95	***

The best model included ACE I/D genotypes, age (younger olds, 80–90 years, and older olds, ≥91 years), sex, and triglyceride blood concentration (<1.70 mmol/l and ≥1.70 mmol/l)

*p=0.01; **p=0.001; ***p=0.006

In order to test the association between ACE I/D polymorphism and longevity, we used previously reported frequencies for the general Croatian population (Barbalić et al. 2004). The genotype distribution in general population was compatible with HWE, but it was not the case in 80+ years population where we found a lack of heterozygotes (p=0.000061, df=1). The ACE genotype (II, ID, and DD) distribution differed significantly between two age cohorts with DD genotype being twice as frequent in senescents as in the younger cohort. D allele frequency was also higher in elderlies (Table 5). Both ACE I/D genotype (p<0.00001) and allele (p<0.001) distribution differences between general and 80+ population were statistically significant. The ACE genotype and allele distributions were not significantly different between our NT and HT senescent groups (p>0.05).

The results of the meta-analysis are presented in Fig. 1. In nine of ten analyzed countries (with the exception of Spain), D allele frequencies were higher in elderlies than in general population; individual ORs were statistically significant as well as the total OR amounting 1.19 (95% CI, 1.08–1.31; Fig. 1). Since the Cochrane Q test result indicated that the heterogeneity was low (I^2<11%, p=0.346), the data were pooled by means of the fixed effect model (Mantel–Haenszel method).

Using Begg's (p>0.788) and Egger's test (p>0.799) as well as by visual inspection of the funnel plot (Fig. 2), we found no evidence for publication bias. The influential analysis revealed that no single study (country) was responsible for the overall significance of the estimates (Fig. 3). After removing an individual study each time and recalculating the combined estimates, the overall estimates as well as the heterogeneity statistics remained nearly unchanged. For the includued studies performed by the same research group, we examined the materials and methods sections and assured that these studies contained no overlapping sets of individuals.

Discussion

Numerous worldwide conducted studies have demonstrated that elevated BP is one of the major risk factors for developing cardiovascular diseases. However, only few have tested the association between candidate genes and CVD risk factors in elderly cohort. This study provides information on the ACE I/D polymorphism, HT, dislypidemia, and BMI in Croatian elderly population. We did not confirm previously reported role of the investigated risk factors to the development of HT in our 80+ years cohort. Probably the most remarkable finding from this study is a detection of significantly more D allele carriers among elderlies than in general population, suggesting that ACE D allele contributes to good health and longevity.

Table 5 ACE I/D genotype and allele frequencies in general and elderly (80–101 years) Croatian population

		Genotype distribution			Allele frequency		Total
		DD	ID	II	D	I	
Croatia — general (n=172)	*N*	38	94	40	170	174	344
	%	22.1	54.7	23.3	49.4	50.6	100
Croatia — elderly (n=301)	*N*	129	110	62	368	234	602
	%	42.8	36.5	20.5	61.1	38.9	100
		χ^2=22.045, df=2			χ^2=12.304, df=1		
		p<0.00001			p<0.001		

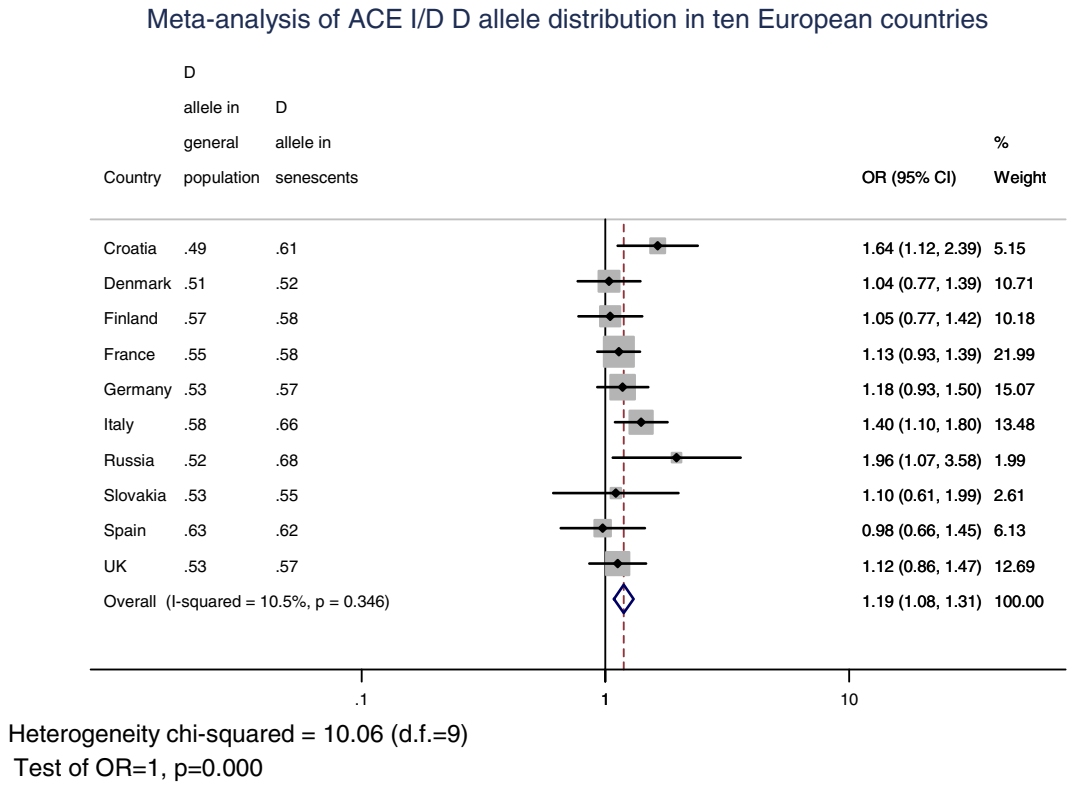

Fig. 1 Forest plot displaying results of the fixed effects meta-analysis of ACE D allele distribution in two age cohorts of ten European countries on a logarithmic scale. Each country data include D allele frequencies in general population and elderlies, partial and overall odds ratios (*ORs*), and their 95% confidence intervals (*CIs*), as well as a contribution of each sample to the overall OR. The overall pooled OR is 1.19 (95% CI, 1.08–1.31, p=0.0003). *Horizontal line* on forest plot represents a 95% CI for each study, and the *size of the square* corresponds to the weight of the study in the meta-analysis. The *solid vertical line* shows an OR of 1, and the *dashed vertical line* corresponds to the overall OR of the sample

The prevalence of HT in our 80+ subjects was thrice as high as the prevalence in Croatian general population 18–64 years (First Croatian Health Project; Ministry of Health of the Republic of Croatia 1995).

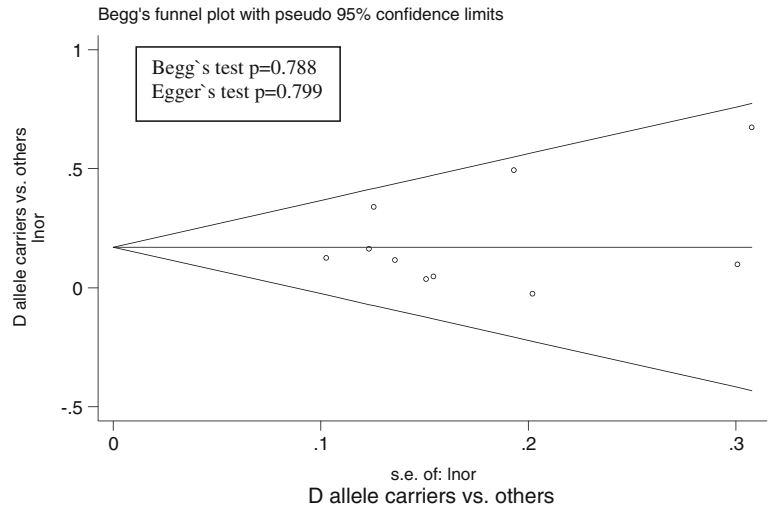

Fig. 2 Funnel plot for the results of meta-analysis of D allele carriers compared to others (non-carriers). The symmetry of the plot indicates no publication or other small studies related bias. The results of the two formal tests for detecting such bias are listed

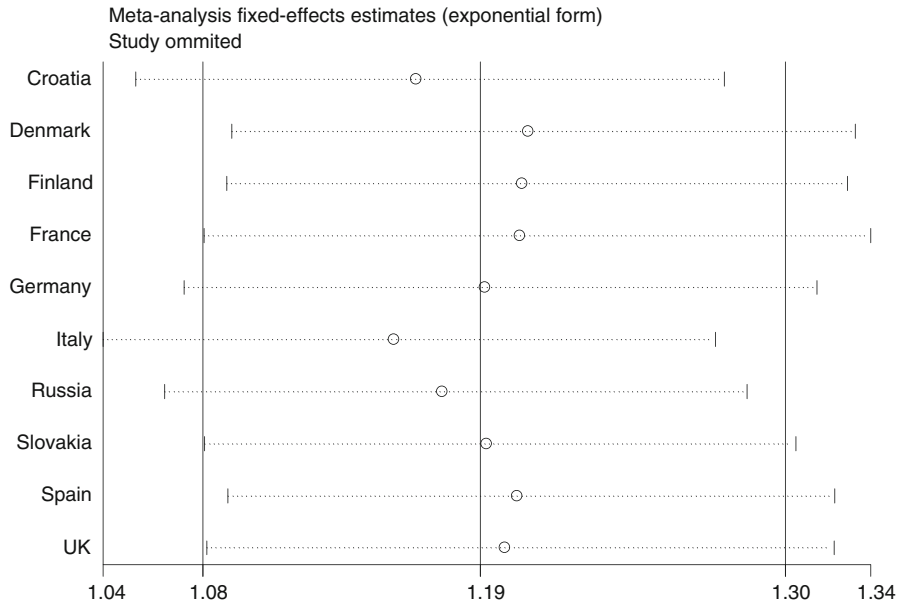

Fig. 3 Influential meta-analysis plot with the effects estimates (ORs) after omiting an individual study each time

It was also higher compared to hypertensives in the elderly Croatian population aged 65–80 years (83% vs. 63%; Škarić-Jurić et al. 2006). The NHANES data, a USA 30-year longitudinal study, presented the growth of prevalence of HT in adults across the age spectrum; 27.3% among participants younger than 60 years of age, 63.0% in those aged 60 to 79 years, and 74.0% in those aged 80 years or older (Lloyd-Jones et al. 2005). These findings are in concordance with pathophysiological changes that occur in the cardiovascular system with aging: decreasing elasticity of the aorta and great arteries, a dropout of myocytes that together with the increased left ventricular (LV) afterload results in modest LV hypertrophy, apoptosis of atrial pacemaker cells, and HT as a consequence of all that (Yin et al. 1982; Vaitkevicius et al. 1993; Cheitlin and Zipes 2001). However, some observations have shown that BP level is closely related to the risk of stroke and heart disease, but the association declines with increasing age (Prospective Studies Collaboration 1995). Considering our finding that HT is more prevalent in women than in men, it is not a surprise that in women, we also detected significantly elevated biochemical risk factors for CVD: total cholesterol, LDL cholesterol, and triglyceride level.

Logistic regression of risk factors for HT showed statistically significant contribution of female sex, triglyceride concentration, and age (younger elderlies, <91 years) to the prevalence of HT. The higher prevalence of HT in women than in men in our study is consistent with the results of Martins et al. (2001), where he concluded that women have higher rates of HT than men as they age. The prevalence of CVD in women is low during the reproductive years and after menopause women's risk rises from two to three times. Carlson et al. (2004) reported that a decrease in estrogen production leads to the development of atherosclerosis. In a recent review, Labreuche et al. (2010) found a positive association between elevated triglyceride levels and stroke and carotid atherosclerosis.

The role of the ACE gene in the pathogenesis of HT is well known and documented (Jeunemaitre et al. 1992a), yet many ACE I/D genotype distribution results in hypertensive vs. nonhypertensive subjects are contradictory (Szadkowska et al. 2006; Freitas et al. 2007; Miyama et al. 2007). Some explain this inconsistency with genetic and environmental heterogeneity between different ethnic groups (Bautista et al. 2004; Zhang et al. 2007; Higaki et al. 2000; Jiménez et al. 2007; Companioni Nápoles et al. 2007) as well as ACE I/D polymorphism gene's complex interaction with other genetic factors that contribute to the expression of HT (Nawaz and Hasnain 2008).

Previously reported results pointed to the association of ACE D polymorphism with HT in general Croatian population (Barbalić et al. 2006), while in 80+ years

population, we did not confirm it. Our findings do not support population-specific, but age-conditioned association of ACE I/D genotype with HT.

The first study of ACE I/D genotype distribution within centenarians and controls, conducted by Schachter et al. (1994), reported unexpectedly frequent occurrence of DD genotype in the French centenarians compared with the control group (40% vs. 26%, $p=0.01$). The experiment was repeated in 2000 with nearly twice that sample, but the results failed to confirm previous findings (Blanche et al. 2001). Neither the Rotterdam study results (Arias-Vasquez et al. 2005) nor the results of the Italian study (Nacmias et al. 2007) suggested any relation of ACE I/D polymorphism with longevity. Then again, Lufts group observed that in German population over 80 years, D allele occurred at higher frequency than in youngs (Luft 1999).

Considering widely investigated role of ACE I/D polymorphism in various diseases, namely, CVD, Alzheimer's disease, sarcoidosis, and others, allele frequencies are reported in numerous studies for different age groups. Since the said longevity studies results were inconclusive, we conducted a meta-analysis in both healthy general and 80+ European populations. In the analysis, we included subjects that represented healthy controls for D allele frequencies in various diseases, as well as the subjects from previously mentioned longevity studies. It is possible that some relevant studies were not included in our review, as we limited our search to reports published in English. With the only exception of Spain, the results from all available countries were similar to ours; ACE gene D allele frequencies were higher in subjects 80+ years than in general adult population.

Schahter's group proposed that the risk of developing CVD conferred by the D allele is redeemed by a possible long-term protective effect; such an effect may give some early selective advantage and/or a late reversal of its negative survival influence. They also suggested a potential relation of protective effect of DD genotype to other biological functions of ACE besides in RAS and kinin–kallikrein systems: neuroendocrine and immunomodulator functions related to ACE levels may contribute to overall survival and longevity (Ehlers and Riordan 1989; McGeer and Singh 1992; Costerousse et al. 1993). The association of DD genotype with longevity may also be derived from linkage disequilibrium to a closely mapping gene still to be identified. In addition, it is interesting to mention that a gene encoding for the human growth hormone also maps to chromosome 17q23, shows strong linkage to ACE, and appears to have an important role in senescence (McGeer and Singh 1992; Crisan and Carr 2000; Huang et al. 2007).

Comparing centenarians and middle-aged controls from Italy, France, and Denmark, Panza et al. (2003) reported a decrease, although statistically insignificant, in ACE I allele frequencies from North to South of Europe in both age groups. In 2004, Barbalić graphically presented the worldwide frequency distribution of ACE Alu insertion, where she reported lowest frequency of I allele in Africa, which increases toward Asia and Australia on one side and the Americas on the other with general Croatian population frequency of 50.06% falling within the range of European populations. We found no geographical gradient of ACE D allele in our meta-analysis, neither in general nor in 80+ populations.

In conclusion, we presented in this study that the ACE D allele was not associated with HT in Croatian senescent population, regardless of its well-known role in BP regulation and direct impact at CVD development. ACE DD genotype and D allele frequency were more frequent among the senescents than in general Croatian population. Such genetic differences, which were confirmed with meta-analysis including other European populations, indicate that D allele, apart from being CVD risk factor in middle–aged population, might have some, yet unrecognized, advantageous role in successful human aging.

Acknowledgment This research was supported by a grant from the Ministry of Science, Education, and Sports of the Republic of Croatia 196-1962766-2747 to N. Smolej Narančić. We would like to thank the study participants and the administration, medical teams, and employees of the homes for elderly and infirm. We are also grateful to Professor Daniela Sivakova for providing us raw data from 80+ years Slovakian population. We also appreciate help from our colleagues and friends, Professor Branka Janićijević and Ana Barešić, PhD student, in collecting field data.

References

Agerholm-Larsen B, Tybjaerg-Hansen A, Frikke-Schmidt R et al (1997) ACE gene polymorphism as a risk factor for ischemic cerebrovascular disease. Ann Intern Med 127:346–355

Agerholm-Larsen B, Nordestgaard BG, Tybjaerg-Hansen A (2000) ACE gene polymorphism in cardiovascular disease: meta-analyses of small and large studies in whites. Arterioscler Thromb Vasc Biol 20:484–492

Alía P, Mañá J, Capdevila O et al (2005) Association between ACE gene I/D polymorphism and clinical presentation and prognosis of sarcoidosis. Scand J Clin Lab Invest 65 (8):691–698

Alvarez R, Alvarez V, Lahoz CH et al (1999) Angiotensin converting enzyme and endothelial nitric oxide synthase DNA polymorphisms and late onset Alzheimer's disease. J Neurol Neurosurg Psychiatry 67:733–736

Arbustini E, Grasso M, Fasani R et al (1995) Angiotensin converting enzyme gene deletion allele is independently and strongly associated with coronary atherosclerosis and myocardial infarction. Br Heart J 74:584–591

Arias-Vasquez A, Sayed-Tabatabaei FA, Schut AF et al (2005) Angiotensin converting enzyme gene, smoking and mortality in a population-based study. Eur J Clin Invest 35:444–449

Barbalić M, Peričić M, Škarić-Jurić T, Smolej Narančić N (2004) ACE Alu insertion polymorphism in Croatia and its isolates. Coll Antropol 28:603–610

Barbalić M, Škarić-Jurić T, Cambien F et al (2006) Gene polymorphisms of the renin–angiotensin system and early development of hypertension. Am J Hypertens 19 (8):837–842

Bautista LE, Ardila ME, Gamarra G, Vargas CI, Arenas IA (2004) Angiotensin-converting enzyme gene polymorphism and risk of myocardial infarction in Colombia. Med Sci Monit 10(8):473–479

Benigni A, Cassis P, Remuzzi G (2010) Angiotensin II revisited: new roles in inflammation, immunology and aging. EMBO Mol Med 2:247–257

Bladbjerg EM, Andersen-Ranberg K, de Maat MP et al (1999) Longevity is independent of common variations in genes associated with cardiovascular risk. Thromb Haemost 82:1100–1105

Blanche H, Cabanne L, Sahbatou M, Thomas G (2001) A study of French centenarians: are ACE and APOE associated with longevity? C R Acad Sci III 324:129–135

Brewster UC, Perazella MA (2004) The renin–angiotensin–aldosterone system and the kidney: effects on kidney disease. Am J Med 116:263–272

Busjahn A, Knoblauch J, Knoblauch M et al (1997) Angiotensin converting enzyme and angiotensinogen gene polymorphisms, plasma levels, and left ventricular size: a twin study. Hypertension 29:165–170

Cambien F, Poirier O, Lecerf L et al (1992) Deletion polymorphism in the gene for angiotensin-converting-enzyme is a potent risk factor for myocardial infarction. Nature 359:641–644

Carlson KJ, Eisenstat SA, Ziporyn T (2004) The new Harvard guide to women's health. Harvard University, Boston, pp 41–42

Cheitlin MD, Zipes DP (2001) Cardiovascular disease in the elderly. In: Braunwald E, Zipes DP, Libby P (eds) Heart disease, 6th edn. WB Saunders, Philadelphia, p 2019

Clarke R, Emberson J, Fletcher A et al (2009) Life expectancy in relation to cardiovascular risk factors: 38 year follow-up of 19 000 men in the Whitehall study. BMJ 339:3513–3521

Companioni Nápoles O, Sautié Castellanos M, Leal L et al (2007) ACE I/D polymorphism study in a Cuban hypertensive population. Clin Chim Acta 378:112–116

Corbo RM, Ulizzi L, Piombo L, Scacchi R (2008) Study on a possible effect of four longevity candidate genes (ACE, PON1, PPAR-c, and APOE) on human fertility. Biogerontology 9:317–323

Costerousse O, Allegrini J, Lopez M, Alhenc-Gelas F (1993) Angiotensin I converting enzyme in human circulating mononuclear cells: genetic polymorphism of expression in T-lymphocytes. Biochem J 290:33–40

Crisan D, Carr J (2000) Angiotensin I-converting enzyme: genotype and disease associations. J Mol Diagn 2:105–115

Croatian Bureau of Statistics (1992) Census 1991. Croatian Bureau of Statistics, Zagreb

Dankova Z, Sivakova D, Luptakova L, Blazicek P (2009) Association of ACE (I/D) polymorphism with metabolic syndrome and hypertension in two ethnic groups in Slovakia. Anthropol Anz 67(3):305–316

de Cavanagh EM, Inserra F, Ferder M, Ferder L (2007) From mitochondria to disease: role of the renin–angiotensin system. Am J Nephrol 27:545–553

Di Pasquale P, Cannizzaro S, Paterna S (2004) Does angiotensin-converting enzyme gene polymorphism affect blood pressure? Findings after 6 years of follow-up in healthy subjects. Eur J Heart Fail 6:11–16

Dikmen M, Günes HV, Degirmenci I, Özdemir G, Basaran A (2006) Are the angiotensin-converting enzyme gene and activity risk factors for stroke? Arq Neuropsiquiatr 64 (2):212–216

Dolgikh MM, Voevoda MI, Malyutina SK (2001) Age change of ACE gene genotypes and alleles frequencies in urban population of West Siberia. First workshop on information technologies application to problems of biodiversity and dynamics of ecosystems in North Eurasia (WITA-2001), Book of abstracts

Egger M, Davey Smith G, Schneider M et al (1997) Bias in meta analysis detected by a simple, graphical test. BMJ 315(7109):629–634

Ehlers MRW, Riordan JF (1989) Angiotensin-converting enzyme: new concepts concerning its biological role. Biochemistry 28:5311–5313

Fleming I (2006) Signaling by the angiotensin-converting enzyme. Circ Res 98:887–896

Freitas SRS, Cabello PH, Moura-Neto RS, Dolinsky LC, Bóia MN (2007) Combined Analysis of genetic and environmental factors on essential hypertension in a Brazilian rural population in the Amazon Region. Arq Bras Cardiol 88:393–397

Fuentes RM, Perola M, Nissinen A, Tuomilehto J (2002) ACE gene and physical activity, blood pressure and hypertension: a population study in Finland. J Appl Physiol 92:2508–2512

Galinsky D, Tysoe C, Brayne CE et al (1997) Analysis of the apo E/apo C-I, angiotensin converting enzyme and methylenetetrahydrofolate reductase genes as candidates affecting human longevity. Atherosclerosis 129:177–183

Garrib A, Zhou W, Sherwood R, Peters T (1998) Angiotensin-converting enzyme (ACE) gene polymorphism in patients with sarcoidosis. Biochem Soc Trans 26:137

Hadjadj S, Tarnow L, Forsblom C et al (2007) Association between angiotensin-converting enzyme gene polymorphisms and diabetic nephropathy: case-control, haplotype, and family-based study in three European populations. J Am Soc Nephrol 18:1284–1291

Hernández Ortega E, Medina Fernández-Aceituno A, Rodríguez-Esparragón FJ et al (2002) The involvement of the renin–angiotension system gene polymorphisms in coronary heart disease. Rev Esp Cardiol 55:92–99

Higaki J, Baba S, Katsuya T et al (2000) Deletion allele of angiotensin-converting enzyme gene in creases risk of essential hypertension in Japanese men: the Suita study. Circulation 101:2060–2065

Higgins JP, Thompson SG, Deeks JJ, Altman DG (2003) Measuring inconsistency in meta-analyses. BMJ 327:557–560

Huang S, Chen XH, Payne JR et al (2007) Haplotype of growth hormone and angiotensin I-converting enzyme genes, serum angiotensin I-converting enzyme and ventricular growth: pathway inference in pharmacogenetics. Pharmacogenet Genomics 17(4):291–294

Islam MS, Lehtimäki T, Juonala M et al (2006) Polymorphism of the angiotensin-converting enzyme (ACE) and angiotesinogen (AGT) genes and their associations with blood pressure and carotid artery intima media thickness among healthy Finnish young adults—the cardiovascular risk in young Finns study. Atherosclerosis 188:316–322

Jackson A, Brown K, Langdown J et al (2000) Effect of the angiotensin-converting enzyme gene deletion polymorphism on the risk of venous thromboembolism. Br J Haematol 111:562–564

Jeunemaitre X, Soubrier F, Kotelevtsev YV et al (1992a) Molecular basis of human hypertension: role of angiotensinogen. Cell 71:169–180

Jeunemaitre X, Lifton RP, Hunt SC, Williams RR, Lalouel JM (1992b) Absence of linkage between the angiotensin converting enzyme locus and human essential hypertension. Nat Genet 1:72–75

Jeunemaitre X, Inoue I, Williams C et al (1997) Haplotypes of angiotensinogen in essential hypertension. Am J Hum Genet 51:1448–1460

Jiménez PM, Conde C, Casanegra A et al (2007) Association of ACE genotype and predominantly diastolic hypertension: a preliminary study. J Renin Angiotensin Aldosterone Syst 8:42–44

Kalaydjieva L, Perez-Lezaun A, Angelicheva D et al (1999) A founder mutation in the GK1 gene is responsible for galactokinase deficiency in Roma (gypsies). Am J Hum Genet 65(5):1299–1307

Keavney BD, Dudley CR, Stratton IM et al (1995) UK prospective diabetes study (UKPDS) 14: association of angiotensin-converting enzyme insertion/deletion polymorphism with myocardial infarction in NIDDM. Diabetologia 38(8):948–952

Kehoe PG, Russ C, McIlory S et al (1999) Variation in DCP1, encoding ACE, is associated with susceptibility to Alzheimer disease. Nat Genet 21:71–72

Kitayama H, Maeshima Y, Takazawa Y et al (2006) Regulation of angiogenic factors in angiotensin II infusion model in association with tubulointerstitial injuries. Am J Hypertens 19:718–727

Labreuche J, Deplanque D, Touboul PJ, Bruckert E, Amarenco P (2010) Association between change in plasma triglyceride levels and risk of stroke and carotid atherosclerosis: systematic review and meta-regression analysis. Atherosclerosis 212 (1):9–15

Lindpaintner K, Pfeffer MA, Kreutz R et al (1995) A prospective evaluation of an angiotensin converting enzyme gene polymorphism and the risk of ischemic heart disease. N Engl J Med 332:706–711

Lloyd-Jones DM, Evans JC, Levy D (2005) Hypertension in adults across the age spectrum: current outcomes and control in the ommunity. JAMA 294(4):466–472

Lopez AD, Mathers CD, Ezzati M, Jamison DT, Murray CJ (2006) Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. Lancet 367(9524):1747–1757

Luft FC (1999) Bad genes, good people, association, linkage, longevity and the prevention of cardiovascular disease. Clin Exp Pharmacol Physiol 26(7):576–579

Marre M, Jeunemaitre X, Gallois Y et al (1997) Contribution of genetic polymorphism in the renin–angiotensin system to the development of renal complications in insulin-dependent diabetes: Genetique de la Nephropathie Diabetique (GENEDIAB) study group. J Clin Invest 99 (7):1585–1595

Martins D, Nelson K, Pan D, Tareen N, Norris K (2001) The effect of gender on age-related blood pressure changes and the prevalence of isolated systolic hypertension among older adults: data from NHANES III. J Gend Specif Med 4 (3):10–13, 20

McGeer EG, Singh EA (1992) Angiotensin-converting enzyme in cortical tissue in Alzheimer's and some other neurological diseases. Dementia 3:299–303

Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from nucleated cells. Nucleic Acids Res 16:1215

Miloserdova OV, Slominsky PA, Limborska SA (2001) Age-dependent variation of the allele and genotype frequencies in insertion–deletion polymorphism for the angiotensin-converting enzyme gene. Genetika 38(1):87–89

Ministry of Health of the Republic of Croatia (1995) First Croatian health project: final report. Ministry of Health, Zagreb

Miyama N, Hasegawa Y, Suzuki M et al (2007) Investigation of major genetic polymorphisms in the renin–angiotensin–aldosterone system in subjects with young-onset hypertension selected by a tar geted-screening system at university. Clin Exp Hypertens 29:61–67

Mondry A, Loh M, Pengbo L, Zhu AL, Nagel M (2005) Polymorphisms of the insertion/deletion ACE and M235T AGT genes and hypertension: surprising new findings and meta-analysis of data. BMC Nephrol 6:1–11

Myllykangas L, Polvikoski T, Sulkava R et al (2000) Cardiovascular risk factors and Alzheimer's disease: a genetic association study in a population aged 85 or over. Neurosci Lett 292:195–198

Nacmias B, Bagnoli S, Tedde A et al (2007) Angiotensin converting enzyme insertion/deletion polymorphism in sporadic and familial Alzheimers disease and longevity. Arch Gerontol Geriatr 45(2):201–206

Nawaz SK, Hasnain S (2008) Pleiotropic effects of ACE polymorphism. Biochemia Med 19(1):36–49

Nazarov IB, Woods DR, Montgomery HE et al (2001) The angiotensin converting enzyme I/D polymorphism in Russian athletes. Eur J Hum Genet 9:797–801

Panza F, Solfrizzi V, D'Introno A et al (2003) Angiotensin I converting enzyme (ACE) gene polymorphism in cente-

narians: different allele frequencies between the North and South of Europe. Exp Gerontol 38:1015–1020
Paolisso G, Tagliamonte MR, De Lucia D et al (2001) ACE gene polymorphism and insulin action in older subjects and healthy centenarians. J Am Geriatr Soc 49:610–614
Papadopoulos KI, Melander O, Orho-Melander M et al (2000) Angiotensin converting enzyme (ACE) gene polymorphism in sarcoidosis in relation to associated autoimmune diseases. J Intern Med 247:71–77
Paterna S, Di Pasquale P, D'Angelo A et al (2000) Angiotensin-converting enzyme gene deletion polymorphism determines an increase in frequency of migraine attacks in patients suffering from migraine without aura. Eur Neurol 43(3):133–136
Petiti DB (1994) Meta-analysis decision analysis and cost-effectiveness analysis, Oxford University Press
Platten M, Youssef S, Hur EM et al (2009) Blocking angiotensin-converting enzyme induces potent regulatory T cells and modulates TH1- and TH17-mediated autoimmunity. Proc Natl Acad Sci USA 106:14948–14953
Prospective Studies Collaboration (1995) Cholesterol, diastolic blood pressure and stroke: 13000 strokes in 450000 people in 45 prospective cohorts. Lancet 346:1647–1653
Pueyo ME, Gonzalez W, Nicoletti A et al (2000) Angiotensin stimulates endothelial vascular cell adhesion molecule-1 via nuclear factor-kappaB activation induced by intracellular oxidative stress. Arterioscler Thromb Vasc Biol 20:645–651
Richard F, Fromentin-David I, Ricolfi F et al (2001) The angiotensin I converting enzyme gene as a susceptibility factor for dementia. Neurology 56:1593–1595
Riera-Fortuny C, Real JT, Chaves FJ et al (2005) The relation between obesity, abdominal fat deposit and the angiotensin-converting enzyme gene I/D polymorphism and its association with coronary heart disease. Int J Obes Relat Metab Disord 29:78–84
Rigat B, Hubert C, Alhenc-Gelas F et al (1990) An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest 86:1343–1346
Samani NJ, O'Toole L, Martin D et al (1996) Insertion/deletion polymorphism in the angiotensin-converting enzyme gene and risk of and prognosis after myocardial infarction. J Am Coll Cardiol 28:338–344
Sayed-Tabatabaei FA, Houwing-Duistermaat JJ, van Duijn CM, Witteman JC (2003) Angiotensin-converting enzyme gene polymorphism and carotid artery wall thickness: a meta-analysis. Stroke 34:1634–1639
Schachter F, Faure-Delanef L, Guenot F et al (1994) Genetic associations with human longevity at the APOE and ACE loci. Nat Genet 6:29–32
Schunkert H, Hense H-W, Holmer S-R et al (1994) Association between a deletion polymorphism of the angiotensin converting enzyme gene and left ventricular hypertrophy. N Engl J Med 330:1634–1638
Sharma P (1998) Meta-analysis of the ACE gene in ischaemic stroke. J Neurol Neurosurg Psychiatry 64:227–230
Sharma P, Smith I, Maguire G, Stewart S, Shneerson J, Brown MJ (1997) Clinical value of ACE genotyping in diagnosis of sarcoidosis. Lancet 349:1602–1603
Sivakova D, Lajdova A, Basistova Z, Cvicelova Z, Blazicek P (2009) ACE insertion/deletion polymorphism and its relationships to the components of metabolic syndrome in elderly Slovaks. Anthropol Anz 67(1):1–11
Škarić-Jurić T, Smolej Narančić N, Barbalić M, et al. (2006) Distribucija čimbenika rizika za kardiovaskularne bolesti u osoba starijih od 65 godina u Republici Hrvatskoj. In: Anic B, Tomek-Roksandic S (Eds.), 2nd Croatian gerontolological congress with international participation: book of abstracts. Liječnički vjesnik 128(1):64–65
Staessen JA, Wang JG, Ginocchio G et al (1997) The deletion/insertion polymorphism of the angiotensin converting enzyme gene and cardiovascular-renal risk. J Hypertens 15:1579–1592
Suzuki Y, Ruiz-Ortega M, Lorenzo O et al (2003) Inflammation and angiotensin II. Int J Biochem Cell Biol 35:881–900
Szadkowska A, Pietrzak I, Klich I et al (2006) Polymorphism I/D of the angiotensin-converting enzyme gene and disturbance of blood pressure in type 1 diabetic children and adolescents. Przegl Lek 63:32–36
Vaisi-Raygani A, Ghaneialvar H, Rahimi Z et al (2010) The angiotensin converting enzyme D allele is an independent risk factor for early onset coronary artery disease. Clin Biochem 43:1189–1194
Vaitkevicius PV, Fleg JL, Engel JH et al (1993) Effects of age and aerobic capacity on arterial stiffness in healthy adults. Circulation 88:1456–1462
Villar J, Flores C, Pérez-Méndez L et al (2008) Angiotensin-converting enzyme insertion/deletion polymorphism is not associated with susceptibility and outcome in sepsis and acute respiratory distress syndrome. Intensive Care Med 34:488–495
Weiner JS, Lourie JA (1981) Practical human biology (International biological programme handbook No. 9). Academic, London
WHO (2000) Obesity: preventing and managing the global epidemic. Report of a WHO Consultation. WHO Technical Report Series 894. World Health Organization, Geneva
Xu X, Li J, Sheng W, Liu L (2008) Meta-analysis of genetic studies from journals published in China of ischemic stroke in the Han Chinese population. Cerebrovasc Dis 26(1):48–62
Yin FCP, Spurgeon HA, Rakusan K, Weisfeldt ML, Lakatta EG (1982) Use of tibial length to quantify cardiac hypertrophy: application in the aging rat. Am J Physiol 243:941–947
Zhang YL, Zhou SX, Lei J, Zhang JM (2007) Association of angiotensin I-converting enzyme gene polymorphism with ACE and PAI-1 levels in Guangdong Chinese Han patients with essential hypertension. Nan Fang Yi Ke Da Xue Xue Bao 27(11):1681–1684