jraas

Journal of the Renin-Angiotensin-Aldosterone System
January-March 2017: 1–7

DOI: 10.1177/1470320316688345
journals.sagepub.com/home/jra
SAGE

The relationship between angiotensin-converting enzyme (ACE) insertion (I) / deletion (D) polymorphism, serum ACE activity and bone mineral density (BMD) in older Chinese

Ya-feng Zhang[1,2], Hong Wang[1], Qiong Cheng[3], Ling Qin[4], Nelson LS Tang[5], Ping-chong Leung[6] and Timothy CY Kwok[2]

Abstract

Objective: In this study, we set out to investigate the relationship between angiotensin-converting enzyme (*ACE*) I/D polymorphism, serum ACE activity and bone mineral density (BMD) in older Chinese.

Methods: A standardized, structured, face-to-face interview was performed to collect demographic information. BMD was measured using dual-energy X-ray absorptiometry (DXA). I/D genotypes of *ACE* were determined by polymerase chain reaction (PCR) amplification. Serum ACE activity was determined photometrically by a commercially available kinetic kit. Multiple linear regression analysis was used to examine the relationship between *ACE* I/D polymorphism, serum ACE activity and BMD.

Results: A total of 1567 males and 1760 females were selected for analyzing the relationship between ACE I/D polymorphism and BMD. There was no significant difference in spine BMD, total hip BMD and femur neck BMD among different *ACE* I/D genotypes both in males and females. A total of 1699 males and 1739 females were selected for analyzing the relationship between serum ACE activity and BMD. There was also no significant difference in spine BMD, total hip BMD and femur neck BMD among different serum ACE activity groups both in males and females.

Conclusion: There was no relationship between *ACE* I/D polymorphism, serum ACE activity and BMD in older Chinese.

Keywords

Angiotensin-converting enzyme, I/D polymorphism, serum ACE activity, bone mineral density, Chinese

Date received: 8 May 2016; accepted: 7 November 2016

Introduction

Angiotensin-converting enzyme (ACE) is a transmembrane zinc metallopeptidase, which has a major role in the metabolism of vasoactive peptides, by converting decapeptide angiotensin I into vasoconstrictor octapeptide angiotensin II and inactivating bradykinin.[1,2] ACE is bound to endothelial surface membrane by an anchor peptide, which could be cleft by ACE secretase to form a soluble enzyme called serum ACE.[3]

In 1990, Rigat et al. found that a polymorphism involving the presence (insertion, I) or absence (deletion, D) of a 287-bp sequence of DNA in intron 16 of the *ACE* gene accounted for approximately half of the variant in serum ACE activity in Caucasians: Individuals with DD genotype had highest serum ACE activity, those with II genotype had

[1]Department of Orthopaedics, The Affiliated Hospital to Nantong University, PR China
[2]Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong SAR, PR China
[3]Jiangsu Provincial Key Laboratory of Neural Regeneration, Nantong University, PR China
[4]Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong, Hong Kong SAR, PR China
[5]Department of Chemical Pathology, The Chinese University of Hong Kong, Hong Kong SAR, PR China
[6]Jockey Club Center for Osteoporosis Care and Control, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong SAR, PR China

Y.Z. and H.W. contributed equally to this study.

Corresponding author:
Timothy C.Y. Kwok, Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Shatin, Hong Kong SAR, PR China.
Email: tkwok@cuhk.edu.hk

the lowest serum ACE activity and those with ID genotype had intermediate serum ACE activity.[4] In Asian individuals, *ACE* I/D polymorphism also had a great impact on serum ACE activity, with the same trend as in Caucasians.[5]

ACE polymorphism was correlated to many diseases; for example, atherosclerosis, coronary heart disease, stroke, diabetic nephropathy, and Alzheimer disease.[6–10] It was also reported that *ACE* polymorphism was associated with muscle performance.[11] In a previous randomized study, it was reported that ACE inhibitor treatment was effective in increasing bone mineral density (BMD) in a subgroup of women with the *ACE* DD genotype,[12] which suggested that *ACE* I/D polymorphism may also correlate with bone metabolism. However, whether there is a relationship between *ACE* I/D polymorphism and BMD still needs further investigation.

It was reported that plasma ACE level determination was more predictive than *ACE* I/D genotype for risk of restenosis after coronary stent, which may due to the large variation of serum ACE activity in different *ACE* I/D genotypes.[13] Although serum ACE activity was significantly affected by *ACE* I/D genotype and the serum ACE activity of the DD genotype was nearly twice as high as that of the II genotype, the variation of serum ACE activity in the DD genotype was still very large; as a categorical variable, *ACE* I/D genotype may be less precise than serum ACE activity since it was a continuous variable.[4,14] The physiological and pathophysiological effects of serum ACE activity are still not fully understood, and since it was reported that ACE inhibitor treatment can increase the BMD in people with the DD genotype,[12] it is interesting to know the relationship between serum ACE activity and bone metabolism.

A previous cross-sectional study showed that ACE inhibitor use was associated with higher BMD in older Chinese, which suggested that angiotensin II may have a detrimental effect on bone; however, in the same cohort study, longitudinal data showed ACE inhibitor use increased bone loss.[15] The longitudinal data from another center of the same multicenter cohort study also showed that ACE inhibitor use was associated with increased bone loss.[16] So the real relationship between the renin angiotensin system (RAS) and BMD still needs further investigation. In this study, we set out to investigate the relationship between *ACE* I/D polymorphism, serum ACE activity and BMD in older Chinese.

Materials and methods

Participants were from Mr.OS-Hong Kong and Ms.OS-Hong Kong, which were set up to investigate the risk factors for osteoporotic fracture in Hong Kong-dwelling older Chinese. The inclusive criteria of Mr.OS-Hong Kong and Ms.OS-Hong Kong were almost similar to those of Mr.OS-US, and designed to result in a cohort of older individuals, which is representative of the communities of Hong Kong.[17] The study population in Hong Kong consisted of community-dwelling, ambulatory people aged 65 years and above. A total of 4000 individuals (2000 of each gender) were recruited using a combination of private solicitation and public advertising from community centers, housing estates, and the general community in Hong Kong. Stratified sampling was employed to ensure that approximately one-third of the participants fell into each of the following age strata: 65–69, 70–74 and 75 and above years old.[18] In this study, people who were taking osteoporosis-related medications, ACE inhibitors and Angiotensin II receptor blockers (ARBs) were excluded from analysis.

A standardized, structured, face-to-face interview was performed to collect demographic information on lifestyle, personal medical history and medication history. Details of information collection methods have been described elsewhere.[17] BMD was measured for proximal femur and lumbar spine vertebrate using dual-energy X-ray absorptiometry (DXA) with Hologic QDR 4500 bone densitometers (Hologic, Waltham, MA, USA). Calibration was performed daily on a lumbar spine phantom, and the coefficient of variation was 0.7%.

Peripheral venous blood was taken after overnight fasting for serum isolation and DNA extraction. DNA was later extracted using a standard phenol/chloroform extraction method. I/D genotypes of ACE were determined by polymerase chain reaction (PCR) amplification. PCR mixtures of 25 μl were set up that contained 1X reaction buffer (Fermentas Life Sciences), 2 mM MgCl2, 1 μM of each forward and reverse primer, 0.2 mM each deoxynucleotide (dNTP), 0.6 U Taq polymerase (Fermentas Life Sciences) and 50 ng DNA. The sequences of forward and reverse primer were 5′-AGAGAGACTCAAGCACGCCC-3′ and 5′-ACCCAAGTGCCAGTGATGTT-3′, respectively. The thermal cycling profile began with initial denaturation at 96°C for five minutes, followed by 35 cycles of 96°C for 30 seconds, 63.8°C for 45 seconds, 72°C for 30 seconds, and a final extension at 72°C for 10 minutes. Amplification products yielded were of 439 bp for the D allele and 727 bp for the I allele. The products were then separated by electrophoresis in 2% agarose gels with ethidium bromide and were visualized under ultraviolet transillumination.

Serum ACE activity was determined photometrically by a commercially available kinetic kit purchased from Bühlmann Laboratories AG (Allschwil, Switzerland). Testing was performed according to the manufacturer's instructions. ACE catalyzes the hydrolysis of the synthetic substance N-[3-(2-furyl)acryloyl]-L-phenylalanylglycylglycine, and this hydrolysis results in a decrease in absorbance at 340 nm. The measurement was automatically performed by Roche COBAS MIRA Plus Chemistry Analyzer.

Participants were divided into three groups according to *ACE* I/D polymorphism: II, ID and DD, or four groups according to the serum ACE activity quartile values. Data of continuous variables were presented as mean (SD) and data

Table 1. Comparison of clinical features of older men among different *ACE* I/D genotypes.

	ACE genotype			*p* value
	II (*n* = 698)	ID (*n* = 717)	DD (*n* = 152)	
Age (years)	72.6 (5.0)	72.1 (4.9)	72.1 (4.8)	0.794
Weight (kg)	62.4 (9.4)	61.7 (8.9)	63.0 (10.5)	0.194
BMI	23.42 (3.12)	23.19 (2.95)	23.44 (3.40)	0.329
PASE total score	97.27 (51.65)	99.20 (52.04)	94.66 (45.03)	0.555
Current smoker	96 (13.8%)	81 (11.3%)	24 (15.8%)	0.199
Hypertension	285 (40.8%)	306 (42.7%)	64 (42.1%)	0.778
COPD	74 (10.6%)	87 (12.1%)	14 (9.2%)	0.475
Coronary heart disease	64 (9.2%)	64 (8.9%)	15 (9.9%)	0.934
Cardiac failure	24 (3.4%)	24 (3.3%)	1 (0.7%)	0.185
Diabetes	84 (12.0%)	81 (11.3%)	18 (11.8%)	0.909
Peripheral vascular disease	26 (3.7%)	32 (4.5%)	8 (5.3%)	0.624
Drug use				
Alpha blocker	79 (11.3%)	69 (9.6%)	15 (9.9%)	0.565
Beta blocker	106 (15.2%)	95 (13.2%)	22 (14.5%)	0.578
Thiazide diuretics	20 (2.9%)	26 (3.6%)	5 (3.3%)	0.543
Statin	35 (5.0%)	25 (3.5%)	8 (5.3%)	0.311
Nitrate	45 (6.4%)	41 (5.7%)	5 (3.3%)	0.318
Inhaled/oral corticosteroid	7 (1.0%)	10 (1.4%)	2 (1.3%)	0.564
Calcium supplement	71 (10.2%)	72 (10.0%)	15 (9.9%)	0.992

Data of continuous variables were presented as mean (SD) and data of categorical variables were presented as number (frequency). Continuous variables were compared by ANOVA test and categorical variables were compared by Chi-square test. *ACE*: angiotensin-converting enzyme; I: insertion; D: deletion; BMI: body mass index; PASE: Physical Activity Scale for Elderly; COPD: chronic obstructive pulmonary disease; ANOVA: analysis of variance.

of categorical variables were presented as numbers (frequency). Continuous variables were compared by analysis of variance (ANOVA) test and categorical variables were compared by Chi-square test. Multiple linear regression analysis was used to examine the relationship between *ACE* I/D polymorphism, serum ACE activity and BMD. Covariates in the regression model was selected according to previous study of the same cohort, which included age, weight, body mass index (BMI), Physical Activity Scale for Elderly (PASE) total score, current smoker, hypertension, diabetes mellitus, coronary heart disease, cardiac failure, chronic obstructive pulmonary disease (COPD), peripheral vascular disease, alpha-blocker, beta-blocker, thiazide diuretics, statin, nitrate, corticosteroid, and calcium supplement use.[17,18] A p value of less than 0.05 was regarded as statistically significant. All the analyses were performed with SPSS software (version 11.0, Chicago, IL, USA).

Results

After excluding antiosteoporosis drug users, ACE inhibitor users, ARB users and those individuals who did not have *ACE* I/D genotype data because of technical problems with blood samples, 1567 males and 1760 females were selected for analyzing the relationship between *ACE* I/D genotype and BMD. Out of 1567 males, 698 were II genotype, 717 were ID genotype and 152 were DD genotype. Out of 1760 females, 810 were II genotype, 753 were ID genotype, and 197 were DD genotype. The clinical characteristics and drug use of males and females are compared in Tables 1 and 2, respectively. There were no significant findings in males or females.

There was no significant difference in spine BMD, total hip BMD and femur neck BMD between different *ACE* I/D genotypes in males or females after adjusting for selected confounders. Details are shown in Table 3.

In total, 1699 males and 1739 females were selected for analyzing the relationship between serum ACE activity and BMD. The quartile values for males and females were 33.6, 44.7, 58.3 and 33.6, 43.3, 56.3, respectively. The clinical characteristics and drug use of males and females are compared in Tables 4 and 5, respectively. There was no significant difference of clinical characteristics and drug use between different serum ACE activity quartile groups in males or females.

After adjusting for selected confounders, there was also no significant difference in spine BMD, total hip BMD and femur neck BMD between different serum ACE activity groups in males and females (Table 6).

Discussion

This cross-sectional study showed that there was no relationship between *ACE* I/D polymorphism and spine, total

Table 2. Comparison of clinical features of older women among different *ACE* I/D genotypes.

	ACE genotype			*p* value
	II (*n* = 810)	ID (*n* = 753)	DD (*n* = 197)	
Age (years)	72.2 (5.3)	72.8 (5.3)	73.3 (6.2)	0.008
Weight (kg)	54.4 (8.3)	54.3 (8.5)	54.7 (8.4)	0.803
BMI	23.85 (3.33)	23.87 (3.53)	23.90 (3.40)	0.984
PASE total score	87.83 (34.66)	84.61 (31.70)	84.18 (34.65)	0.116
Current smoker	11 (1.4%)	19 (2.5%)	4 (2.0%)	0.212
Hypertension	327 (40.4%)	325 (43.2%)	88 (44.7%)	0.392
COPD	40 (4.9%)	36 (4.8%)	10 (5.1%)	0.981
Coronary heart disease	67 (8.3%)	69 (9.2%)	13 (6.6%)	0.497
Cardiac failure	29 (3.6%)	23 (3.1%)	9 (4.6%)	0.569
Diabetes	101 (12.5%)	99 (13.1%)	26 (13.2%)	0.911
Peripheral vascular disease	58 (7.2%)	73 (9.7%)	16 (8.1%)	0.190
Drug use				
Alpha blocker	3 (0.4%)	6 (0.8%)	5 (2.5%)	0.006
Beta blocker	128 (15.8%)	115 (15.3%)	32 (16.2%)	0.929
Thiazide diuretics	61 (7.5%)	58 (7.7%)	8 (4.1%)	0.191
Statin	39 (4.8%)	43 (5.7%)	9 (4.6%)	0.670
Nitrate	44 (5.4%)	36 (4.8%)	12 (6.1%)	0.716
Inhaled/oral corticosteroid	9 (1.1%)	6 (0.8%)	4 (2.0%)	0.579
Calcium supplement	140 (17.3%)	125 (16.6%)	40 (20.3%)	0.473

Data of continuous variables were presented as mean (SD) and data of categorical variables were presented as number (frequency). Continuous variables were compared by ANOVA test and categorical variables were compared by Chi-square test. ACE: angiotensin-converting enzyme; I: insertion; D: deletion; BMI: body mass index; PASE: Physical Activity Scale for Elderly; COPD: chronic obstructive pulmonary disease; ANOVA: analysis of variance.

Table 3. Comparison of BMD of older individuals among different I/D genotypes.

	ACE genotype			*p* value1	*p* value2
	II	ID	DD		
Male	*n* = 698	*n* = 717	*n* = 152		
Spine BMD	0.94 (0.17)	0.94 (0.18)	0.94 (0.17)	0.931	0.822
Total hip BMD	0.86 (0.12)	0.86 (0.13)	0.86 (0.13)	0.441	0.410
Femur neck BMD	0.68 (0.10)	0.68 (0.11)	0.68 (0.11)	0.886	0.858
Female	n=810	n=753	n=197		
Spine BMD	0.75 (0.14)	0.75 (0.15)	0.77 (0.15)	0.182	0.370
Total hip BMD	0.71 (0.12)	0.70 (0.12)	0.71 (0.12)	0.312	0.745
Femur neck BMD	0.59 (0.10)	0.58 (0.10)	0.58 (0.10)	0.207	0.478

Multiple linear regression analysis was used to examine the relationship between *ACE* I/D polymorphism and BMD. Covariates in the regression model included age, weight, BMI, PASE total score, current smoker, hypertension, diabetes mellitus, coronary heart disease, cardiac failure, COPD, peripheral vascular disease, alpha-blocker, beta-blocker, thiazide diuretics, statin, nitrate, corticosteroid, and calcium supplement use. (*p* value1: unadjusted *p* value, *p* value2: *p* value adjusted for confounders). BMD: bone mineral density; I: insertion; D: deletion; *ACE*: angiotensin-converting enzyme; PASE: Physical Activity Scale for Elderly.

hip and femur neck BMD in male and female older Chinese individuals.

There were experimental data to suggest that the RAS has an influence on bone health. Angiotensin II directly stimulates DNA and collagen synthesis in osteoblasts[19,20] and stimulates osteoclasts when they are co-cultured with osteoblasts.[21] It may also influence bone cell metabolism by its effect on blood flow in bone microvasculature, or via its effects on calcium metabolism.[22] Lower ACE activity in bone may also lead to a local rise in bradykinin levels, resulting in local vasodilatation.[23] This may have a beneficial effect on BMD.[24–26]

Previously, a small cross-sectional study showed that the *ACE* II genotype was associated with higher lumbar spine BMD in postmenopausal women.[27] However, another study found that the DD genotype was associated with higher BMD at the lumbar spine in renal failure patients on hemodialysis.[28] In a prospective trial of ACE inhibitors in female

Table 4. Comparison of clinical features and drug use of older men between different serum ACE activity quartile groups.

	Serum ACE activity quartile groups				*p* value
	1 (*n* = 426)	2 (*n* = 424)	3 (*n* = 425)	4 (*n* = 424)	
Serum ACE activity (U/l)	<33.6	33.6–44.7	44.7–58.3	>58.3	
Age (years)	72.1 (4.9)	72.1 (5.0)	72.5 (5.2)	72.3 (5.1)	0.656
Weight (kg)	62.0 (9.1)	61.8 (9.4)	62.3 (8.7)	61.8 (9.9)	0.838
BMI	23.40 (2.97)	23.24 (3.19)	23.27 (2.87)	23.06 (3.20)	0.432
PASE total score	97.89 (51.89)	98.24 (48.74)	97.80 (52.30)	98.88 (52.29)	0.990
Current smoker	46 (0.8%)	69 (16.3%)	49 (11.5%)	49 (11.6%)	0.062
Hypertension	182 (42.7%)	184 (43.4%)	177 (41.6%)	174 (41.0%)	0.900
COPD	47 (11.0%)	52 (12.3%)	46 (10.8%)	48 (11.3%)	0.918
Coronary heart disease	38 (8.9%)	28 (6.6%)	49 (11.5%)	36 (8.5%)	0.091
Cardiac failure	17 (4.0%)	10 (2.4%)	14 (3.3%)	12 (2.8%)	0.564
Diabetes	53 (12.4%)	41 (9.7%)	53 (12.5%)	52 (12.3%)	0.514
Peripheral vascular disease	16 (3.8%)	17 (4.0%)	17 (4.0%)	23 (5.4%)	0.618
Drug use					
Alpha blocker	53 (12.4%)	46 (10.8%)	49 (11.5%)	45 (10.6%)	0.837
Beta blocker	61 (14.3%)	51 (12.0%)	66 (15.5%)	66 (15.6%)	0.416
Thiazide diuretics	12 (2.8%)	16 (3.8%)	17 (4.0%)	12 (2.8%)	0.679
Statin	25 (5.9%)	15 (3.5%)	19 (4.5%)	13 (3.1%)	0.187
Nitrate	23 (5.4%)	22 (5.2%)	32 (7.5%)	21 (5.0%)	0.346
Inhaled/oral corticosteroid	5 (1.2%)	5 (1.2%)	3 (0.7%)	7 (1.7%)	0.683
Calcium supplement	40 (9.4%)	40 (9.4%)	49 (11.5%)	43 (10.1%)	0.706

Data of continuous variables were presented as mean (SD) and data of categorical variables were presented as number (frequency). Continuous variables were compared by ANOVA test and categorical variables were compared by Chi-square test. ACE: angiotensin-converting enzyme; I: insertion; D: deletion; BMI: body mass index; PASE: Physical Activity Scale for Elderly; COPD: chronic obstructive pulmonary disease; ANOVA: analysis of variance.

Table 5. Comparison of clinical features and drug use of older women between different serum ACE activity quartile groups.

	Serum ACE activity quartile groups				*p* value
	1 (*n* = 435)	2 (*n* = 438)	3 (*n* = 433)	4 (*n* = 433)	
Serum ACE activity (U/l)	<33.6	33.6–43.3	43.3–56.3	>56.3	
Age (years)	72.3 (5.2)	72.8 (5.4)	72.3 (5.0)	72.8 (5.9)	0.241
Weight (kg)	54.3 (8.2)	54.0 (8.2)	54.9 (8.7)	54.2 (8.6)	0.384
BMI	23.96 (3.33)	23.74 (3.27)	24.10 (3.56)	23.67 (3.59)	0.220
PASE total score	87.44 (31.97)	87.02 (35.72)	86.47 (32.33)	85.76 (33.08)	0.891
Current smoker	11 (2.5%)	6 (1.4%)	10 (2.3%)	5 (1.2%)	0.343
Hypertension	183 (42.1%)	194 (44.3%)	180 (41.6%)	176 (40.6%)	0.732
COPD	29 (6.7%)	14 (3.2%)	20 (4.6%)	24 (5.5%)	0.115
Coronary heart disease	35 (8.0%)	39 (8.9%)	42 (9.7%)	35 (8.1%)	0.799
Cardiac failure	14 (3.2%)	15 (3.4%)	16 (3.7%)	14 (3.2%)	0.978
Diabetes	53 (12.2%)	47 (10.7%)	61 (14.1%)	58 (13.4%)	0.461
Peripheral vascular disease	32 (7.4%)	37 (8.4%)	35 (8.1%)	40 (9.2%)	0.790
Drug use					
Alpha blocker	3 (0.7%)	3 (0.7%)	3 (0.7%)	6 (1.4%)	0.291
Beta blocker	62 (14.3%)	71 (16.2%)	70 (16.2%)	70 (16.2%)	0.821
Thiazide diuretics	27 (6.2%)	27 (6.2%)	38 (8.8%)	34 (7.9%)	0.361
Statin	23 (5.3%)	27 (6.2%)	21 (4.8%)	21 (4.8%)	0.800
Nitrate	27 (6.2%)	21 (4.8%)	24 (5.5%)	23 (5.3%)	0.833
Inhaled/oral corticosteroid	8 (1.8%)	3 (0.7%)	2 (0.5%)	7 (1.6%)	0.697
Calcium supplement	69 (15.9%)	72 (16.4%)	79 (18.2%)	83 (19.2%)	0.541

Data of continuous variables were presented as mean (SD) and data of categorical variables were presented as number (frequency). Continuous variables were compared by ANOVA test and categorical variables were compared by Chi-square test. ACE: angiotensin-converting enzyme; I: insertion; D: deletion; BMI: body mass index; PASE: Physical Activity Scale for Elderly; COPD: chronic obstructive pulmonary disease; ANOVA: analysis of variance.

Table 6. Comparison of BMD of older individuals between different serum ACE activity quartile groups.

	Serum ACE activity quartile groups				*p* value1	*p* value2
	1	2	3	4		
Male	*n* = 426	*n* = 424	*n* = 425	*n* = 424		
Spine BMD	0.95 (0.19)	0.93 (0.17)	0.93 (0.17)	0.95 (0.17)	0.327	0.812
Total hip BMD	0.86 (0.13)	0.86 (0.13)	0.86 (0.12)	0.86 (0.13)	0.964	0.417
Femur neck BMD	0.68 (0.11)	0.69 (0.11)	0.68 (0.11)	0.69 (0.11)	0.676	0.312
Female	*n* = 435	*n* = 438	*n* = 433	*n* = 433		
Spine BMD	0.74 (0.14)	0.74 (0.15)	0.76 (0.15)	0.76 (0.15)	0.253	0.083
Total hip BMD	0.70 (0.10)	0.71 (0.12)	0.71 (0.12)	0.71 (0.12)	0.383	0.444
Femur neck BMD	0.58 (0.10)	0.58 (0.10)	0.59 (0.10)	0.58 (0.10)	0.679	0.512

Multiple linear regression analysis was used to examine the relationship between serum ACE activity and BMD. Covariates in the regression model included age, weight, BMI, PASE total score, current smoker, hypertension, diabetes mellitus, coronary heart disease, cardiac failure, COPD, peripheral vascular disease, alpha-blocker, beta-blocker, thiazide diuretics, statin, nitrate, corticosteroid, and calcium supplement use. (*p* value1: unadjusted *p* value, *p* value2: *p* value adjusted for confounders). BMD: bone mineral density; ACE: angiotensin-converting enzyme; PASE: Physical Activity Scale for Elderly; COPD: chronic obstructive pulmonary disease; BMI: body mass index.

hypertensive patients for one year, those individuals with the DD genotype had a significant gain in lumbar spine BMD, while women with the other genotypes did not.[12] Hip BMD and males were not examined in these studies.

To date, this is the first study to examine the relationship between *ACE* I/D genotypes and BMD in a large unselected group of older Chinese. It was surprising that the results showed no significant difference in BMD between different *ACE* I/D polymorphism groups, as it was previously reported that ACE inhibitor use was associated with higher BMD in older Chinese.[18]

Based on the average and SD of the total hip BMD of the II genotype group, a group sample size of 150 (as in DD genotype groups in this analysis) should have a power of 80% to detect an average group difference of 0.04 g/cm^2 at a *p* value of 0.05 level in both sexes, which is similar to the average BMD difference between ACE inhibitor users and non-users at baseline.[18] Therefore, the chance of a type one error in detecting a clinically significant difference among the genotype groups was small.

An alternative explanation may be that the *ACE* I/D polymorphism was a categorical variable. Although it has a great impact on serum ACE activity, the variation of serum ACE activity among the same *ACE* genotype group was still very large,[4,14] and this variation may confuse the effect of the *ACE* I/D polymorphism on BMD.

In this study, we also examined the relationship between serum ACE activity and BMD. To our surprise, no significant relationship between serum ACE activity and BMD was found in males or females.

A previous study from the same cohort has shown that ACE inhibitor use was associated with higher BMD both in males and females.[18] In the same cohort, when grouping the participants by *ACE* I/D polymorphism, it was found that there was no significant difference in BMD between different *ACE* I/D polymorphisms. Although the *ACE* I/D polymorphism had a great impact on serum ACE activity, the inter-individual variation of serum ACE activity in the same *ACE* I/D genotype is still very large,[4,14] and this may attenuate the significance of the relationship between *ACE* I/D polymorphism and BMD. So if we subgroup the participants by serum ACE activity, it should provide better significance on BMD than the *ACE* I/D polymorphism.

However, in this study we failed to find a relationship between serum ACE activity and BMD in men and women. As to why there was no significant relationship between serum ACE activity and BMD, the possible reason may be as follows. Although a previous in vitro study showed that angiotensin II was a stimulator of osteoclastic bone resorption,[20] a subsequent study demonstrated that angiotensin II indirectly promoted the activation of osteoclasts via upregulation of receptor activator of nuclear factor-kappa B ligand (RANKL) in osteoblasts;[29] all these results come from in vitro studies. The relationship between the RAS and BMD may be weak, and intersubject variation of angiotensin II level may not result in significant BMD difference.

To sum up, there was no relationship between *ACE* I/D polymorphism, serum ACE activity and BMD in male or female participants. Combining all these results, it can be speculated that the relationship between RAS and BMD may be weak. In order to clarify the relationship between *ACE* I/D polymorphism, serum ACE activity and bone health, further prospective studies may be needed to provide more conclusive information.

The strengths of this study were the large sample size, non-selective nature of the participants and the adjustment for a wide range of confounders. The limitations may be that the sample was confined to older Chinese and was cross-sectional in nature.

Acknowledgements

The authors would like to thank all the participants for their dedicated contribution to the study. The authors also would like to thank Mr Jason Leung and Mr Anthony Kwok from the Jockey Club Centre for Osteoporosis Care and Control, The Chinese University of Hong Kong, for their kind help in this study.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was partially supported by the Research Grants Council Earmarked Grant CUHK 4101/02 M, the National Institutes of Health R01 grant AR049439-01A1, Natural Science Fund of Jiangsu Province, PR China (grant no. BK2010285, BK20151275), Collegiate Natural Science Fund of Jiangsu Province, PR China (grant no. 10KJB320015), China Postdoctoral Science Foundation (grant no. 2013M540459), and the National Natural Science Foundation of China (grant no. 81501913).

References

1. Erdös EG and Skidgel RA. The angiotensin I-converting enzyme. *Lab Invest* 1987; 56: 345–348.
2. Jaspard E, Wei L and Alhenc-Gelas F. Differences in the properties and enzymatic specificities of the two active sites of angiotensin I-converting enzyme (kininase II). Studies with bradykinin and other natural peptides. *J Biol Chem* 1993; 268: 9496–9503.
3. Ramchandran R, Sen GC, Misono K, et al. Regulated cleavage-secretion of the membrane-bound angiotensin-converting enzyme. *J Biol Chem* 1994; 269: 2125–2130.
4. Rigat B, Hubert C, Alhenc-Gelas F, et al. 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: 1343–1346.
5. Wong TY, Szeto CC, Chow KM, et al. Contribution of gene polymorphisms in the renin-angiotensin system to macroangiopathy in patients with diabetic nephropathy. *Am J Kidney Dis* 2001; 38: 9–17.
6. Sayed-Tabatabaei FA, Houwing-Duistermaat JJ, van Duijn CM, et al. Angiotensin-converting enzyme gene polymorphism and carotid artery wall thickness: A meta-analysis. *Stroke* 2003; 34: 1634–1639.
7. Staessen JA, Wang JG, Ginocchio G, et al. The deletion/insertion polymorphism of the angiotensin converting enzyme gene and cardiovascular-renal risk. *J Hypertens* 1997; 15 (12 Pt 2): 1579–1592.
8. Cambien F, Poirier O, Lecerf L, et al. Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. *Nature* 1992; 359: 641–644.
9. Sharma P. Meta-analysis of the ACE gene in ischaemic stroke. *J Neurol Neurosurg Psychiatry* 1998; 64: 227–230.
10. Kehoe PG, Russ C, McIlory S, et al. Variation in DCP1, encoding ACE, is associated with susceptibility to Alzheimer disease. *Nat Genet* 1999; 21: 71–72.
11. Montgomery HE, Marshall R, Hemingway H, et al. Human gene for physical performance. *Nature* 1998; 393: 221–222.
12. Pérez-Castrillón JL, Justo I, Silva J, et al. Bone mass and bone modelling markers in hypertensive postmenopausal women. *J Hum Hypertens* 2003; 17: 107–110.
13. Ribichini F, Steffenino G, Dellavalle A, et al. Plasma activity and insertion/deletion polymorphism of angiotensin I-converting enzyme: A major risk factor and a marker of risk for coronary stent restenosis. *Circulation* 1998; 97: 147–154.
14. Alhenc-Gelas F, Weare JA, Johnson RL Jr, et al. Measurement of human converting enzyme level by direct radioimmunoassay. *J Lab Clin Med* 1983; 101: 83–96.
15. Zhang YF, Qin L, Leung PC, et al. The effect of angiotensin converting enzyme inhibitor use on bone loss in older Chinese. *J Bone Miner Metab* 2012; 30: 666–673.
16. Kwok T, Leung J, Zhang YF, et al. Does the use of ACE inhibitors or angiotensin receptor blockers affect bone loss in older men? *Osteoporos Int* 2012; 23: 2159–2167.
17. Lau EM, Leung PC, Kwok T, et al. The determinants of bone mineral density in Chinese men—results from Mr. Os (Hong Kong), the first cohort study on osteoporosis in Asian men. *Osteoporos Int* 2006; 17: 297–303.
18. Lynn H, Kwok T, Wong SY, et al. Angiotensin converting enzyme inhibitor use is associated with higher bone mineral density in elderly Chinese. *Bone* 2006; 38: 584–588.
19. Hiruma Y, Inoue A, Hirose S, et al. Angiotensin II stimulates the proliferation of osteoblast-rich populations of cells from rat calvariae. *Biochem Biophys Res Commun* 1997; 230: 176–178.
20. Hatton R, Stimpel M and Chambers TJ. Angiotensin II is generated from angiotensin I by bone cells and stimulates osteoclastic bone resorption in vitro. *J Endocrinol* 1997; 152: 5–10.
21. Hagiwara H, Hiruma Y, Inoue A, et al. Deceleration by angiotensin II of the differentiation and bone formation of rat calvarial osteoblastic cells. *J Endocrinol* 1998; 156: 543–550.
22. Saavedra JM, Benicky J and Zhou J. Mechanisms of the anti-ischemic effect of Angiotensin II AT(1) receptor antagonists in the brain. *Cell Mol Neurobiol* 2006; 26: 1099–1111.
23. Xu J, Carretero OA, Shesely EG, et al. The kinin B1 receptor contributes to the cardioprotective effect of angiotensin-converting enzyme inhibitors and angiotensin receptor blockers in mice. *Exp Physiol* 2009; 94: 322–329.
24. Gustafson GT and Lerner U. Bradykinin stimulates bone resorption and lysosomal-enzyme release in cultured mouse calvaria. *Biochem J* 1984; 219: 329–332.
25. Lerner UH. Bradykinin synergistically potentiates interleukin-1 induced bone resorption and prostanoid biosynthesis in neonatal mouse calvarial bones. *Biochem Biophys Res Commun* 1991; 175: 775–783.
26. Lerner UH, Jones IL and Gustafson GT. Bradykinin, a new potential mediator of inflammation-induced bone resorption. Studies of the effects on mouse calvarial bones and articular cartilage in vitro. *Arthritis Rheum* 1987; 30: 530–540.
27. Woods D, Onambele G, Woledge R, et al. Angiotensin-I converting enzyme genotype-dependent benefit from hormone replacement therapy in isometric muscle strength and bone mineral density. *J Clin Endocrinol Metab* 2001; 86: 2200–2204.
28. Altun B, Kiykim AA, Seyrantepe V, et al. Association between activated renin angiotensin system and bone formation in hemodialysis patients: Is the bone mass genetically determined by *ACE* gene polymorphism? *Ren Fail* 2004; 26: 425–431.
29. Shimizu H, Nakagami H, Osako MK, et al. Angiotensin II accelerates osteoporosis by activating osteoclasts. *FASEB J* 2008; 22: 2465–2475.