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

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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

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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 clef 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

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

$ACE$ polymorphism was correlated to many diseases; for example, atherosclerosis, coronary heart disease, stroke, diabetic nephropathy, and Alzheimer disease.\textsuperscript{6–10} It was also reported that $ACE$ polymorphism was associated with muscle performance.\textsuperscript{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,\textsuperscript{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.\textsuperscript{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.\textsuperscript{5,14} 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,\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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 MgCl$_2$, 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'-AGAGAGACTCAAGCACGCC-3' and 5'-ACCCCAAGTGCAGTGATGT-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 Bühmann 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
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. 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).

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

| ACE genotype | II (n = 698) | ID (n = 717) | DD (n = 152) | p value |
|--------------|-------------|-------------|-------------|---------|
| 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.

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 quartile 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
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 and stimulates osteoclasts when they are co-cultured with osteoblasts. It may also influence bone cell metabolism by its effect on blood flow in bone microvasculature, or via its effects on calcium metabolism. Lower ACE activity in bone may also lead to a local rise in bradykinin levels, resulting in local vasodilatation. This may have a beneficial effect on BMD.

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

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

| ACE genotype | II (n = 810) | ID (n = 753) | DD (n = 197) | p value |
|--------------|--------------|--------------|--------------|---------|
| 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 | II (n = 698) | ID (n = 717) | DD (n = 152) | p value1 | p value2 |
|--------------|--------------|--------------|--------------|----------|----------|
| Male         |              |              |              |          |          |
| 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       |              |              |              |          |          |
| 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 and stimulates osteoclasts when they are co-cultured with osteoblasts. It may also influence bone cell metabolism by its effect on blood flow in bone microvasculature, or via its effects on calcium metabolism. Lower ACE activity in bone may also lead to a local rise in bradykinin levels, resulting in local vasodilatation. This may have a beneficial effect on BMD.

Previously, a small cross-sectional study showed that the ACE II genotype was associated with higher lumbar spine BMD in postmenopausal women. However, another study found that the DD genotype was associated with higher BMD at the lumbar spine in renal failure patients on hemodialysis. 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 | 1 (n = 426) | 2 (n = 424) | 3 (n = 425) | 4 (n = 424) | p value |
|-----------------------------------|-------------|-------------|-------------|-------------|---------|
| 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 | 1 (n = 435) | 2 (n = 438) | 3 (n = 433) | 4 (n = 433) | p value |
|-----------------------------------|-------------|-------------|-------------|-------------|---------|
| 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.
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. 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.

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/cm² 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. 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, and this may attenuate the significance of the relationship between ACE I/D polymorphism and 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. 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, and this may confuse the significance of the relationship between ACE I/D polymorphism and BMD. So if we sub-group 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 that the sample was confined to older Chinese and was cross-sectional in nature.

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.

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