Association between ACE gene I/D polymorphism and knee osteoarthritis in a Chinese population

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Osteoarthritis (OA) is a degenerative joint disease characterized by joint destruction with cartilage loss and occasional gross derangement of joint integrity. In recent years, several studies have reported the association between angiotensin-converting enzyme (ACE) gene insertion/deletion (I/D) polymorphism and knee OA. However, the results were conflicting. To determine the association between ACE gene I/D polymorphism and knee OA, we conducted a hospital-based case–control study with 282 knee OA cases and 316 controls to investigate the association between ACE gene I/D polymorphism and knee OA susceptibility in a Chinese Han population. The present study found that DD genotype or D allele carriers of ACE gene I/D polymorphism increased the risk of knee OA. Stratification analyses of sex, age, and body mass index (BMI) showed significant associations amongst the groups of females, ≥55 years, and abnormal BMI. In addition, the present study made analysis between ACE I/D polymorphism and some clinical features of OA, and found DD genotype of I/D polymorphism was associated with arthralgia. Furthermore, we undertook a meta-analysis together with the present study between this single nucleotide polymorphism (SNP) and knee OA risk. This meta-analysis found that ACE gene I/D polymorphism was associated with increased risk for OA. Stratification analysis of ethnicity in this meta-analysis indicated that I/D polymorphism increased the risk of knee OA amongst the Asians and Caucasians. In conclusion, this case–control study and meta-analysis suggest that ACE gene I/D polymorphism is associated with increased risk for knee OA.

Introduction

Osteoarthritis (OA) is a degenerative joint disorder resulting in destruction of articular cartilage, osteophyte formation, and subchondral bone sclerosis [1]. OA is a polygenic disease and its pathogenesis is influenced by several environmental factors such as ageing, hormones, mechanical factors, and lifestyle. Genetic factors account for 50% of the risk of OA development [2] and prior research suggests that OA is primarily influenced by genetic risk factors due to common population polymorphisms in multiple genes [3–6]. Therefore, candidate gene studies may provide insight into OA development.

Angiotensin-converting enzyme (ACE) gene is localized on chromosome 17 and contains a polymorphism based on the presence (insertion, I) or absence (deletion, D) within intron 16, of a 287-bp Alu repeat sequence within intron 16, resulting in three different genotypes: DD and II homozygous and ID heterozygous. Plasma ACE levels vary with polymorphism. The role of ACE polymorphism has been investigated as a risk factor for several diseases such as inflammatory and immune-related disorders [7], particularly the rheumatic and autoimmune diseases.

Several studies have investigated the relationship between OA and the ACE I/D polymorphism [7–12], but the results were contradictory. As gene pools, lifestyle, and gene–environment interactions vary amongst the populations, the risk shall not be supposed as identical in every population with respect to genotypes. Therefore, we conducted this case–control study to investigate the association between ACE
gene I/D polymorphism and knee OA susceptibility in a Chinese Han population. In addition, we performed a meta-analysis together with the present study to verify the relationship between this single nucleotide polymorphism (SNP) and knee OA risk comprehensively.

**Materials and methods**

**Study population**

A hospital-based case–control design was used in the present study. A total of 282 knee OA patients and 316 healthy controls were selected from Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College between February 2014 and May 2017. The diagnosis of knee OA fulfilled the American College of Rheumatology criteria (1987) [13]. Inclusion criteria were: (i) any symptom and/or sign of knee OA, (ii) no evidence for any other form of arthritis, and (iii) informed consent obtained. Participants who had undergone knee surgery, and those with any systemic inflammatory or autoimmune disorder, or any type of malignant or chronic illness were excluded from the present study. Control subjects were consecutively selected amongst people without personal and family history of knee OA and were matched for age (±5 years) and sex. The demographic, lifestyle, and clinical characteristics of all patients and controls, such as age, gender, body mass index (BMI: kg/m²), and K-L grade were collected from medical records. This case–control study was approved by the Ethics Committee of Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College and performed according to Declaration of Helsinki. All patients provided written informed consent prior to participation.

**Genomic DNA extraction and genotyping**

Peripheral blood samples were taken from all patients and controls, and stored in EDTA tubes. DNA was extracted from blood examples using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). The genotypes of ACE gene polymorphism were determined by PCR using primers and conditions described previously [8,12]. The PCR program included the following steps: initial denaturation at 95°C for 5 min; 35 cycles of denaturation at 95°C for 30s, annealing at 58°C for 30 s, and extension at 72°C for 30s and a final extension at 72°C for 10 min. To control the quality of genotyping, two independent investigators interpreted images of each gel, and at least 10% of samples were randomly selected for repeated genotyping.

**Statistical analysis**

Hardy–Weinberg equilibrium (HWE) of the SNP genotypes was analyzed by the goodness-of-fit chi-square (χ²) test to compare the observed and expected genotype frequencies amongst controls. Association between ACE gene I/D polymorphism and knee OA risk was assessed by logistic regression with odds ratio (OR) and 95% confidence interval (CI). χ² test was used to assess the difference in the demographic characteristics, variables, and the genotypes of the ACE gene I/D polymorphism. Above statistical analyses were performed on SAS software package (ver. 9.1.3; SAS Institute, Cary, NC, U.S.A.) with the significant level at P<0.05. To fully investigate the association of ACE gene I/D polymorphism with knee OA, we also conducted a meta-analysis which was performed using the Stata 11.0 software (StataCorp, College Station, TX, U.S.A.).

**Results**

**Clinical information of the study population**

The characteristics of the subjects in the case and control groups are summarized in Table 1. Cases and controls were well matched in terms of age and gender (P=0.593 and P=0.328, respectively), and no significant differences in gender and age were observed between the OA patients and controls. A significant association was found in the subgroup of BMI. In addition, disease severity in the knee OA cases and controls was assessed via the Kellgren-Lawrence grade (K-L grade). The controls and cases were concentrated in grade 0–4. Other characteristics of the subjects are presented in Table 1.

**Association between ACE gene I/D polymorphism and knee OA risk**

The genotype distributions of ACE gene I/D polymorphism in all subjects are delineated in Table 2. Genotype distributions for rs2292832 polymorphism in the controls conformed to the HWE (P=0.802). Logistic regression analyses revealed that the DD genotype or D allele carriers of I/D polymorphism was significantly associated with increased risk of knee OA (DD compared with ID+II: adjusted OR = 1.83, 95% CI = 1.15–2.37, P=0.007; ID+DD compared with II: adjusted OR = 1.65, 95% CI = 1.15–2.37, P=0.007; D compared with I: OR = 1.47, 95% CI = 1.16–1.85,
Table 1 Characteristics of subjects with knee OA and control subjects

| Variable                  | Patients (n=282) | Controls (n=316) | P-value |
|---------------------------|------------------|------------------|---------|
| Age (years)               | 54.34 ± 4.55     | 54.62 ± 4.54     | 0.500   |
| Number of male/female     | 75/207           | 77/239           | 0.532   |
| BMI (kg/m²)               | 27.42 ± 3.34     | 25.00 ± 3.44     | <0.001  |
| Onset age                 | 46.48 ± 6.52     |                  |         |
| Duration of OA            | 7.56 ± 4.16      |                  |         |
| ESR, mm/h                 | 13.65 ± 4.83     | 14.20 ± 5.00     | 0.174   |
| CRP, mg/l                 | 2.50 ± 0.89      | 1.66 ± 0.46      | <0.001  |

Symptoms of patients

- Arthralgia: 219 (77.7%)
- Arthroncus: 186 (66.0%)
- Muscle atrophy: 31 (11.0%)
- Joint deformity: 23 (8.2%)

Kellgren–Lawrence grade

- 1: 62 (22.0%)
- 2: 141 (50.0%)
- 3: 65 (23.0%)
- 4: 14 (5.0%)

Abbreviations: CRP, C-reaction protein; ESR, erythrocyte sedimentation rate.

Table 2 Logistic regression analysis of associations between ACE I/D polymorphism and risk of knee OA

| Genotype | Cases¹ (n=282) | Controls² (n=316) | OR (95% CI) | P       | Adjusted OR (95% CI)² | Adjusted P² |
|----------|----------------|-------------------|-------------|---------|-----------------------|------------|
| II       | 39             | 67                | 1.0         |         |                       |            |
| ID       | 128            | 157               | 1.00 (0.89, 2.22) | 0.150  | 1.55 (0.95, 2.54) | 0.077      |
| DD       | 112            | 91                | 2.11 (1.31, 3.42) | 0.002  | 2.28 (1.37, 3.82) | 0.002      |
| DD compared with ID+II    | 112/167        | 91/214            | 1.00 (0.89, 2.22) | 0.150  | 1.55 (0.95, 2.54) | 0.077      |
| ID+DD compared with II    | 240/39         | 248/67            | 1.66 (1.08, 2.56) | 0.021  | 1.65 (1.15, 2.37) | 0.007      |

Allele frequency

- I: 206 (36.9%)
- D: 352 (63.1%)

Bold values are statistically significant (P<0.05).

¹The genotyping was successful in 279 cases and 315 controls.
²Adjusted for age, sex, and BMI.

P=0.001; Table 2). We also conducted stratification analyses of sex, age, and BMI. We obtain significant associations amongst the groups of females, ≥55 years, abnormal BMI (Table 3). Furthermore, we investigated the association between ACE I/D polymorphism and clinical features of OA and found DD genotype of this SNP was associated with arthralgia (Table 4).

Results of false-positive report probability analysis for significant findings

We preset 0.2 as the false-positive report probability (FPRP) threshold. As shown in Table 5, at the prior probability of 0.1, the significant findings for the ACE I/D polymorphism remained unstable in the allele, dominant, homozygote, and heterozygote models. Moreover, the same effects were also seen in the stratification analyses of population-based control (PB), HWE-positive, and Asian groups.

General characteristics of included studies and quantitative analysis of this meta-analysis

Table 6 lists the characteristics of included studies exploring the associations between ACE gene I/D polymorphism and knee OA. Four Asian studies (including the present study), two Caucasian studies, and one Arabian study were
Table 3 Stratified analyses between ACE I/D polymorphisms and the risk of OA

| Variable | Case/control | ID compared with II | DD compared with II | DD compared with ID+II | DD+ID compared with II |
|----------|--------------|---------------------|---------------------|------------------------|------------------------|
|          | II           | ID                  | DD                  | ID+II                  | DD+ID                  |
|          |              | OR (95% CI); P       | OR (95% CI); P       | OR (95% CI); P         | OR (95% CI); P         |
| Sex      |              |                      |                     |                        |                        |
| Male     | 13/14        | 35/34                | 26/28               | 1.10 (0.46, 2.70); 0.821| 1.00 (0.40, 2.52); 1.000| 0.93 (0.48, 1.81); 0.828| 1.06 (0.46, 2.44); 0.892|
| Female   | 26/53        | 93/123               | 86/63               | 1.54 (0.90, 2.65); 0.117| 2.78 (1.57, 4.92); <0.001| 2.02 (1.35, 3.01); <0.001| 1.96 (1.18, 3.27); 0.010|
| Age (years) |            |                      |                     |                        |                        |                        |
| <55      | 18/32        | 65/76                | 61/52               | 1.52 (0.78, 2.96); 0.217| 2.09 (1.05, 4.14); 0.036| 1.53 (0.96, 2.43); 0.076| 1.75 (0.93, 3.29); 0.081|
| ≥55      | 21/35        | 63/81                | 51/39               | 1.30 (0.69, 2.44); 0.422| 2.18 (1.10, 4.31); 0.025| 1.81 (1.09, 2.99); 0.021| 1.58 (0.87, 2.88); 0.133|
| BMI      |              |                      |                     |                        |                        |                        |
| Normal   | 5/22         | 17/55                | 14/34               | 1.36 (0.45, 4.14); 0.588| 1.81 (0.57, 5.74); 0.313| 1.44 (0.66, 3.15); 0.360| 1.53 (0.53, 4.40); 0.427|
| Abnormal | 34/45        | 111/102              | 98/57               | 1.44 (0.86, 2.42); 0.169| 2.28 (1.31, 3.95); 0.004| 1.74 (1.17, 2.60); 0.006| 1.74 (1.06, 2.84); 0.027|

Bold values are statistically significant (P<0.05).

Table 4 The association between ACE I/D polymorphism and clinical features of OA

| Variables | Genotype | Heterozygote | Homozygote | Dominant | Recessive |
|-----------|----------|--------------|------------|----------|-----------|
|           |          | OR (95% CI); P | OR (95% CI); P | OR (95% CI); P | OR (95% CI); P |
| Onset age | ≤52      | 89            | 101        | 31       | 1.0       |
|           | >52      | 23            | 27         | 8        | 1.04 (0.43, 2.51); 0.938 | 1.00 (0.41, 2.47); 0.998 | 1.02 (0.44, 2.36); 0.964 | 0.98 (0.54, 1.76); 0.932 |
| Arthralgia| -        | 23            | 30         | 15       | 1.0       |
|           | +        | 89            | 98         | 24       | 1.82 (0.78, 4.21); 0.161 | 2.43 (1.02, 5.78); 0.042 | 2.07 (0.94, 4.60); 0.068 | 1.52 (0.85, 2.72); 0.158 |
| Arthroncus| -        | 41            | 41         | 14       | 1.0       |
|           | +        | 71            | 87         | 25       | 1.19 (0.56, 2.52); 0.653 | 0.97 (0.45, 2.07); 0.937 | 1.08 (0.53, 2.19); 0.833 | 0.85 (0.52, 1.41); 0.527 |
| Muscle atrophy | -  | 99            | 111        | 38       | 1.0       |
|           | +        | 13            | 17         | 1        | 5.82 (0.75, 45.22); 0.059 | 5.00 (0.63, 39.47); 0.094 | 5.43 (0.72, 41.01); 0.067 | 1.09 (0.51, 2.32); 0.829 |
| Joint deformity | -  | 101           | 118        | 37       | 1.0       |
|           | +        | 11            | 10         | 2        | 1.57 (0.33, 7.48); 0.570 | 2.02 (0.43, 9.52); 0.368 | 1.77 (0.40, 7.88); 0.446 | 1.41 (0.60, 3.31); 0.433 |
| K-L grade | 1–2      | 84            | 92         | 26       | 1.0       |
|           | 3–4      | 28            | 36         | 13       | 0.78 (0.36, 1.69); 0.532 | 0.67 (0.30, 1.47); 0.314 | 0.82 (0.40, 1.70); 0.593 | 1.25 (0.72, 2.14); 0.427 |

Bold values are statistically significant (P<0.05).

identified in this meta-analysis. The Newcastle–Ottawa Scales (NOS) scores of all included studies ranged from 5 to 6 stars, suggesting that they were studies of high methodological quality.

Our meta-analysis indicated that ACE gene I/D polymorphism was associated with the increased risk for knee OA (D compared with I: OR = 1.39, 95% CI = 1.03–1.86, P=0.031; DD+ID compared with II: OR = 1.70, 95% CI = 1.11–2.60, P=0.016; DD compared with II: OR = 1.84, 95% CI = 1.02–3.30, P=0.041; ID compared with II: OR = 1.59, 95% CI = 1.11–2.27, P=0.010; Table 7 and Figure 1). In the subgroup analysis of ethnicity, we found that this SNP increased OA risk amongst Asian and Caucasian populations (Table 7 and Figure 2). Stratification analysis by types of OA indicated that ACE gene I/D polymorphism increased the risk of knee OA and unclear OA (Table 7 and Figure 3). We also observed positive results in the Chinese groups. We did not obtain any different conclusions after eliminating the studies which did not meet the HWE, indicating that the data of this meta-analysis were trustworthy and stable.
Table 5 FPRP values for associations between the ACE I/D polymorphism and OA risk

| Variables | OR (95% CI) | P-value | Power | Prior probability |
|-----------|-------------|---------|-------|------------------|
|           |             |         |       | 0.25 | 0.1 | 0.01 | 0.001 | 0.0001 |
| D vs. I   | 1.38 (1.03, 1.86) | 0.034   | 0.708 | 0.229 | 0.471 | 0.908 | 0.990 | 0.999 |
| Caucasian | 2.12 (1.67, 2.68) | <0.001  | 0.622 | <0.001 | <0.001 | 0.002 | 0.015 | 0.133 |
| PB        | 1.52 (1.02, 2.27) | 0.041   | 0.474 | 0.355 | 0.622 | 0.948 | 0.995 | 0.999 |
| Unclear OA| 2.12 (1.67, 2.68) | <0.001  | 0.622 | <0.001 | <0.001 | 0.002 | 0.015 | 0.133 |
| DD+ID compared with II | 1.70 (1.11, 2.60) | 0.014 | 0.500 | 0.285 | 0.545 | 0.929 | 0.993 | 0.999 |
| Caucasian | 3.84 (2.17, 6.78) | <0.001  | 0.500 | 0.001 | 0.003 | 0.028 | 0.222 | 0.741 |
| PB        | 1.98 (1.10, 3.54) | 0.021   | 0.500 | 0.125 | 0.300 | 0.825 | 0.979 | 0.998 |
| Unclear OA| 3.84 (2.17, 6.78) | <0.001  | 0.500 | <0.001 | <0.001 | 0.001 | 0.007 | 0.067 |
| DD compared with ID+II | 1.52 (1.02, 2.27) | 0.034 | 0.622 | <0.001 | <0.001 | <0.001 | 0.001 | 0.005 |
| Caucasian | 4.32 (2.48, 7.54) | <0.001  | 0.500 | <0.001 | <0.001 | <0.001 | 0.001 | 0.005 |
| Unclear OA| 4.32 (2.48, 7.54) | <0.001  | 0.500 | <0.001 | <0.001 | <0.001 | 0.001 | 0.005 |
| HWE-negative | 0.44 (0.21, 0.92) | 0.029   | 0.500 | 0.127 | 0.304 | 0.828 | 0.980 | 0.998 |
| Other methods | 3.40 (1.36, 8.47) | 0.009   | 0.500 | 0.052 | 0.140 | 0.642 | 0.948 | 0.995 |
| ID compared with II | 1.59 (1.11, 2.27) | 0.011 | 0.500 | 0.079 | 0.204 | 0.739 | 0.966 | 0.997 |
| Asian     | 1.26 (1.02, 1.55) | 0.028   | 0.500 | 0.211 | 0.445 | 0.898 | 0.899 | 0.999 |
| Caucasian | 3.26 (2.11, 5.06) | <0.001  | 0.500 | <0.001 | <0.001 | <0.001 | 0.001 | 0.003 |
| HWE-positive | 1.26 (1.02, 1.55) | 0.028   | 0.500 | 0.211 | 0.445 | 0.898 | 0.899 | 0.999 |
| HWE-negative | 2.46 (1.10, 5.49) | <0.001  | 0.500 | 0.150 | 0.346 | 0.853 | 0.983 | 0.999 |
| PB        | 1.79 (1.09, 2.92) | 0.020   | 0.500 | 0.124 | 0.298 | 0.824 | 0.979 | 0.998 |
| Knee OA   | 1.24 (1.01, 1.52) | 0.038   | 0.500 | 0.234 | 0.479 | 0.910 | 0.990 | 0.999 |
| Unclear OA| 3.26 (2.11, 5.06) | <0.001  | 0.500 | <0.001 | <0.001 | <0.001 | 0.001 | 0.003 |

Table 6 Characteristics of included studies

| Author and year | Case | Control | Sex (male/female) | SOC | Country  | Ethnicity | OA TYPE | Case/control | Genotyping method | HWE | NOS |
|-----------------|------|---------|------------------|-----|----------|-----------|---------|--------------|-------------------|-----|-----|
| Hong, 2003      | 58.6 ± 9.4 | N/A     | 48/94 | N/A | PB       | Korea     | Asian   | Knee OA      | PCR               | 0.117 | 6   |
| Shehab, 2008    | 57.07 ± 9.15 | N/A     | 13/102 | N/A | HB        | Kuwait    | Arabian | Knee OA      | PCR               | <0.001 | 5   |
| Bayram, 2010    | 54.16 ± 1.20 | 44.8 ± 2.00 | 38/102 | 17/43 | PB       | Turkey    | Caucasian | Unclear OA  | PCR               | 0.013 | 5   |
| Inair, 2013     | 58.04 ± 10.87 | 53.03 ± 12.88 | 60/161 | 65/135 | PB       | Turkey    | Caucasian | Unclear OA  | PCR               | 0.003 | 6   |
| Poornima, 2014  | 42.41 ± 8.11 | 42.17 ± 7.98 | 32/68  | 31/69  | PB       | India     | Asian   | Knee OA      | PCR               | 0.480 | 5   |
| Lin, 2016       | 74.9 ± 7.1 | 73.3 ± 6.6 | 194/253 | 206/217 | PB       | Taiwan    | Asian   | Knee OA      | PCR               | 0.945 | 6   |
| The present study | 54.34 ± 4.55 | 54.62 ± 5.45 | 75/207 | 77/239 | HB       | China     | Asian   | Knee OA      | MALDI-TOF-MS     | 0.963 | 6   |

Abbreviations: HB, hospital-based control; SOC, source of control.

Discussion
The present case–control study showed that the DD genotype or D carriers of I/D polymorphism was associated with significantly increased risk of knee OA in a Chinese Han population. Additionally, the present study obtained significant associations amongst the groups of females, ≥ 55 years, and abnormal BMI. Furthermore, the study found DD genotype of I/D polymorphism was associated with arthralgia. Using meta-analysis, we also found that ACE gene I/D polymorphism was associated with increased risk of OA. Similar results were replicated in the stratification analyses of ethnicity, HWE, SOC, and types of OA. In conclusion, this case–control study and meta-analysis found that ACE gene I/D polymorphism is associated with increased risk for knee OA.
### Table 7 Meta-analysis of association between ACE I/D polymorphism and the risk of knee OA

| Comparison       | Category     | OR (95% CI) | P-value | P for heterogeneity |
|------------------|--------------|-------------|---------|---------------------|
| **D compared with I** | Total        | 1.38 (1.03, 1.86) | 0.031   | <0.001              |
| Ethnicity        | Asian        | 1.26 (0.91, 1.75) | 0.171   | <0.001              |
|                  | Arabian      | 0.80 (0.53, 1.22) | 0.302   | N/A                 |
|                  | Caucasian    | 2.12 (1.67, 2.68) | <0.001  | 0.484               |
| Population       | Non-Chinese  | 1.45 (0.90, 2.33) | 0.128   | <0.001              |
|                  | Chinese      | 1.26 (0.94, 1.68) | 0.122   | 0.057               |
| HWE              | HWE-positive | 1.26 (0.91, 1.75) | 0.171   | 0.001               |
|                  | HWE-negative | 1.58 (0.85, 2.96) | 0.148   | <0.001              |
| SOC              | PB           | 1.52 (1.02, 2.27) | 0.042   | 0.180               |
|                  | HB           | 1.11 (0.62, 2.00) | 0.719   | 0.152               |
| Types of OA      | Knee OA      | 1.16 (0.86, 1.57) | 0.321   | 0.091               |
|                  | Unclear OA   | 2.12 (1.67, 2.68) | <0.001  | <0.001              |
| **DD+ID compared with II** | Total       | 1.70 (1.11, 2.60) | 0.016   | <0.001              |
| Ethnicity        | Asian        | 1.33 (0.97, 1.83) | 0.078   | 0.097               |
|                  | Arabian      | 0.83 (0.42, 1.62) | 0.578   | N/A                 |
|                  | Caucasian    | 3.84 (2.17, 6.78) | <0.001  | 0.223               |
| Population       | Non-Chinese  | 1.90 (0.94, 3.82) | 0.073   | <0.001              |
|                  | Chinese      | 1.32 (1.05, 1.66) | 0.016   | 0.220               |
| HWE              | HWE-positive | 1.33 (0.97, 1.83) | 0.078   | 0.097               |
|                  | HWE-negative | 2.45 (0.86, 6.93) | 0.093   | 0.001               |
| SOC              | PB           | 1.98 (1.10, 3.54) | 0.022   | 0.359               |
|                  | HB           | 1.23 (0.63, 2.43) | 0.548   | 0.161               |
| Types of OA      | Knee OA      | 1.25 (0.93, 1.68) | 0.141   | 0.054               |
|                  | Unclear OA   | 3.84 (2.17, 6.78) | <0.001  | 0.066               |
| **DD compared with ID+II** | Total       | 1.84 (1.02, 3.30) | 0.041   | <0.001              |
| Ethnicity        | Asian        | 1.27 (0.72, 2.22) | 0.406   | 0.001               |
|                  | Arabian      | 0.78 (0.45, 1.34) | 0.365   | N/A                 |
|                  | Caucasian    | 1.84 (1.20, 2.84) | <0.001  | 0.771               |
| Population       | Non-Chinese  | 1.37 (0.79, 2.4)  | 0.264   | <0.001              |
|                  | Chinese      | 1.26 (0.73, 2.19) | 0.407   | 0.041               |
| HWE              | HWE-positive | 1.27 (0.72, 2.22) | 0.408   | 0.001               |
|                  | HWE-negative | 1.43 (0.80, 2.56) | 0.228   | 0.024               |
| SOC              | PB           | 1.42 (0.85, 2.36) | 0.184   | 0.267               |
|                  | HB           | 1.17 (0.56, 2.44) | 0.877   | 0.230               |
| Types of OA      | Knee OA      | 1.15 (0.71, 1.86) | 0.562   | 0.233               |
|                  | Unclear OA   | 1.91 (1.34, 2.72) | <0.001  | <0.001              |
| **DD compared with II** | Total        | 1.84 (1.02, 3.30) | 0.041   | <0.001              |
| Ethnicity        | Asian        | 1.45 (0.75, 2.82) | 0.268   | 0.001               |
|                  | Arabian      | 0.78 (0.39, 1.56) | 0.484   | N/A                 |
|                  | Caucasian    | 4.32 (2.48, 7.54) | <0.001  | 0.265               |
| Population       | Non-Chinese  | 2.05 (0.84, 5.02) | 0.117   | <0.001              |
|                  | Chinese      | 1.49 (0.75, 2.93) | 0.253   | 0.039               |
| HWE              | HWE-positive | 1.45 (0.75, 2.82) | 0.268   | 0.001               |
|                  | HWE-negative | 2.60 (0.79, 8.55) | 0.115   | <0.001              |
| SOC              | PB           | 2.14 (0.95, 4.83) | 0.066   | 0.737               |
|                  | HB           | 1.33 (0.50, 3.51) | 0.569   | 0.403               |
| Types of OA      | Knee OA      | 1.29 (0.73, 2.28) | 0.377   | 0.322               |
|                  | Unclear OA   | 4.32 (2.48, 7.54) | <0.001  | 0.038               |
| **DD compared with ID** | Total        | 1.59 (1.11, 2.27) | 0.010   | 0.007               |
| Ethnicity        | Asian        | 1.26 (1.02, 1.55) | 0.034   | 0.794               |
|                  | Arabian      | 1.01 (0.42, 2.41) | 0.983   | N/A                 |
|                  | Caucasian    | 3.26 (2.11, 5.06) | <0.001  | 0.326               |
| Population       | Non-Chinese  | 1.81 (1.01, 3.23) | 0.046   | 0.006               |
|                  | Chinese      | 1.29 (1.01, 1.64) | 0.040   | 0.673               |

Continued over
Table 7 Meta-analysis of association between ACE I/D polymorphism and the risk of knee OA (Continued)

| Comparison Category | Category   | Studies | OR (95% CI)       | P-value | P for heterogeneity |
|---------------------|------------|---------|-------------------|---------|---------------------|
| HWE                 | HWE-positive | 4       | 1.26 (1.02, 1.55) | 0.034   | 0.794               |
|                     | HWE-negative | 3       | 2.46 (1.10, 5.49) | 0.028   | 0.038               |
| SOC                 | PB         | 5       | 1.79 (1.09, 2.92) | 0.021   | 0.229               |
|                     | HB         | 2       | 1.30 (1.12, 2.27) | 0.199   | <0.001              |
| Types of OA         | Knee OA    | 5       | 1.24 (1.01, 1.52) | 0.039   | <0.001              |
|                     | Unclear OA | 2       | 3.26 (2.11, 5.06) | <0.001  | <0.001              |

Abbreviations: HB, hospital-based control; SOC, source of control.

Figure 1. Forest plot shows OR for the associations between ACE gene I/D polymorphism and OA risk (D compared with I)

In previous studies, ACE gene was found associated with rheumatic and autoimmune diseases. OA is an inflammatory disorder, which is associated with these proinflammatory cytokines [14]. Angiotensin II played a vital role in OA progression by modulating the synthesis of proinflammatory cytokines including tumor necrosis factor-alpha (TNF-α), CCL2 (C-C motif chemokine ligand 2), and IL-6 (Interleukin-6) [15]. Notably, levels of ACE in synovial fluid were significantly higher than control groups [16] in OA patients. Studies have demonstrated that the presence of D allele of I/D polymorphism is associated with higher levels of plasma ACE [17].

Several studies investigated the association between ACE gene I/D polymorphism and OA risk. Hong et al. [7] found ACE I/D polymorphism was a risk factor for early-onset knee OA in a Korean study. They indicated that I allele was associated with the radiographically severe and functionally poor OA [7]. In a subsequent study from Kuwait, Shehab et al. [12] revealed that there was no significant difference in the distribution of genotype or allele frequency between control subjects and OA groups. In addition, they did not find an association between any genotypes of I/D and clinical severity of OA [12]. Shehab et al. [12] showed a very high incidence of D-allele in Kuwaiti Arabs, while the Korean study [7] indicated the frequency of I-allele was significantly higher in early onset OA. The distinct distribution of allele frequency may explain the different findings of abovementioned two studies [7,12]. Poornima et al. [11] observed that I/D polymorphism was associated with increased risk of OA in an Indian population. Similar results were also obtained in two Turkish studies [8,9]. They [8,9] both suggested that DD genotype of the ACE gene I/D polymorphism increased the risk of knee OA in Turkish populations. Due to the conflicting findings, Lin et al. [10] conducted a case–control study and meta-analysis to validate the association of I/D polymorphism with OA risk. They did not find any significant association between I/D polymorphism and knee OA in the case–control study and meta-analysis [10]. It was noteworthy that they regarded the Turkish populations as Arubians. We thought that the
Figure 2. Stratification analysis by ethnicity shows OR for the association between ACE gene I/D polymorphism and OA risk (D compared with I).

Figure 3. Stratification analysis by type of OA shows OR for the association between ACE gene I/D polymorphism and OA risk (DD+ID compared with II).
Turkish populations should be divided into Caucasians. Moreover, the meta-analysis by Lin et al. [10] actually found a significant difference in the dominant model [OR: 1.72 (95% CI: 1.02 ± 2.90)], indicating that DD/ID genotype carriers had an increased risk for OA [10]. However, Lin et al. [10] regarded it as negative finding. Two previous meta-analyses investigated the association between this SNP and OA risk. Ai et al. [18] only found I/D polymorphism was associated with increased risk for Caucasians, but not in overall populations and Asians. In another meta-analysis, Shang et al. [19] suggested that ID genotype was weakly associated with OA risk. This meta-analysis (total 1447 cases and 1345 controls) indicated that ACE gene I/D polymorphism was associated with the increased risk of OA.

In the subgroup analysis of ethnicity, we found that ACE gene I/D polymorphism increased OA risk amongst Asian and Caucasian populations. However, no significant associations were obtained in the meta-analysis by Lin et al. [10]. In the meta-analysis of Ai et al. [18], they showed significant association only amongst Caucasians, which was different from that of this meta-analysis. This meta-analysis found that SNP was associated with increased risk for overall populations, including Caucasians and Asians. We also conducted the stratification analysis of types of OA, indicating that ACE gene I/D polymorphism increased the knee OA and unclear OA. In addition, we did not obtain any different conclusions after eliminating the studies which did not meet the HWE. Sensitivity analysis indicated that our results were more robust and stable. Furthermore, we performed stratification analyses of sex, age, and BMI, and found significant associations amongst the groups of females, ≥55 years, abnormal BMI, indicating that these exposure factors may play an important role in the interaction between this SNP and OA. We further made analysis between ACE I/D polymorphism and some clinical features of OA and found DD genotype of this SNP was associated with arthralgia.

Several potential limitations of this case–control study and meta-analysis should be considered. First, we cannot conduct the subgroups of some confounding factors due to the lack of corresponding data. Second, our results were based on unadjusted estimates for confounding factors. Third, the sample size of the present study was not large enough, which might make our work underpowered. Fourth, the sample sizes of Caucasians and Arabians were not large enough in this meta-analysis. Fifth, included studies were only involved in Asians, Caucasians, and Arabians, and studies amongst other racial groups are urgently needed. Sixth, the present study only focused on one gene. Seventh, the present study with limited sample sizes could not provide sufficient evidence to draw a certain conclusion. Finally, we cannot conclude that I/D polymorphisms are susceptibility loci for other types of OA, highlighting the necessity for the further investigation of more types of OA.

In conclusion, this case–control study and meta-analysis found that ACE gene I/D polymorphism is associated with increased risk for knee OA. Further multi-center, well-designed studies with larger sample sizes that include gene–environment interactions assessment are warranted to confirm our findings.

Competing interests
The authors declare that there are no competing interests associated with the manuscript.

Author contribution
Conceived and designed the experiments: G.C., S.H., Z.L., and B.Q. Performed the experiments and meta-analysis: B.Q. and G.C. Analyzed the data: S.H. and Z.L. Contributed reagents/materials/analysis tools: S.H. and Z.L. Wrote the paper: B.Q. and G.C.

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Abbreviations
ACE, angiotensin-converting enzyme; BMI, body mass index; CCL2, C-C motif chemokine ligand 2; CI, confidence interval; D, deletion; HWE, Hardy–Weinberg equilibrium; I, insertion; IL-6, Interleukin-6; K-L grade, Kellgren-Lawrence grade; OA, osteoarthritis; OR, odds ratio; PB, population-based control; SNP, single nucleotide polymorphism; TNF-α, tumor necrosis factor-alpha.

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