Title: Effect of rs4646994 polymorphism of angiotensin converting enzyme on the risk of nonischemic cardiomyopathy

Running head: ACE rs4646994 polymorphism and nonischemic cardiomyopathy

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Abstract

**Background:** ACE gene polymorphisms have recently been shown to be associated with risk of developing left ventricular hypertrophy (LVH). However, the results were controversial. We aimed to conduct this meta-analysis to further confirm the association between ACE rs4646994 polymorphism and HCM/DCM.

**Methods:** PubMed, EMBASE, the Chinese national knowledge information database, and Wanfang databases were searched for eligible studies. The Newcastle-Ottawa Scale was used to evaluate the quality of included studies. Then we evaluated the association between ACE gene mutation and HCM/DCM by calculating odds ratios and 95% confidence intervals. Subgroup analysis was further performed to explore situations in specialized subjects. Sensitivity analysis and publication bias was assessed to confirm the study reliability.

**Results:** There were 13 studies on DCM (2004 cases and 1376 controls) and 16 studies on HCM (2161 controls and 1192 patients). ACE rs4646994 polymorphism was significantly associated with DCM in all genetic models. However, in HCM, four genetic models (allele model, homozygous model, heterozygous model and dominant model) showed significant association between ACE rs4646994 polymorphism and DCM. In subgroup analysis, we found that ACE rs4646994 polymorphism was significantly associated with DCM / HCM in Asian population. Finally, we also conducted a cumulative meta-analysis, which indicates that the results of our meta-analysis are highly reliable.

**Conclusion:** ACE rs4646994 polymorphism increases the risk of DCM / HCM in Asian, but not in Caucasian. More case-control studies are needed to strengthen our conclusions and to assess the gene-gene and gene-environment interactions between ACE rs4646994 polymorphism and DCM /
HCM.

**Key words:** ACE, rs4646994, DCM, HCM, meta-analysis
Introduction

Cardiomyopathy is a kind of heterogeneous myocardial disease, resulting from pathological changes in the myocardium of different etiologies, manifesting as ventricular hypertrophy or dilation. Myocardial dysfunction due to other cardiovascular diseases is not part of the spectrum of the disease, such as valvular heart disease, hypertensive heart disease, congenital heart disease, coronary heart disease, or congenital heart disease. It can eventually lead to progressive heart failure, arrhythmia, thromboembolism and sudden death, and has a poor prognosis.

Cardiomyopathy can be generally classified into hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), restrictive cardiomyopathy (RCM), arrhythmogenic right ventricular cardiomyopathy (ARVC), and left ventricular noncompaction (LVNC). Among them, hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM) are the main types of cardiomyopathy. Many previous clinical studies recognized that cardiomyopathy has a familial origin, suggesting that genetic factors may play a crucial role in disease pathogenesis.

Hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM) are caused by mutant sarcomeric genes. Mutations in sarcomeric protein genes can cause changes in myofilament tension that determine cardiac hypertrophy and dilation. Polymorphisms including genes encoding components of the renin-angiotensin (RAS), such as ACE, have recently been shown to be associated with the risk of developing left ventricular hypertrophy (LVH) and thus may influence the clinical phenotype of HCM/DCM. The angiotensin converting enzyme (ACE) gene is located on chromosome 17q23 and is characterized by a major insertion/deletion (rs4646994) polymorphism, consisting of a 289 base pair Alu repeat sequence present or absent in intron 16.
ACE-D/D genotype and thus may be a genetic factor in the pathogenesis of HCM/DCM\textsuperscript{12,13}.

Over the past 20 years, numerous studies have reported the association of insertion/deletion polymorphisms of the angiotensin I converting enzyme gene (ACE rs4646994) with HCM and DCM. But their results are inconsistent, especially the association with DCM, which is currently controversial\textsuperscript{14}. Therefore, we conducted this meta-analysis to further confirm the association between ACE rs4646994 polymorphism and HCM/DCM.

**Method**

We followed PRISMA guidelines (http://prisma-statement.org/) in conducting the systematic review and meta-analysis.

**Search strategy**

As of March 2021, we have used the terms "angiotensin converting enzyme" or "ACE", "polymorphism" or "mutation" and "hypertrophic cardiomyopathy" and "dilated cardiomyopathy" without language restrictions in PubMed, EMBASE, the Chinese national knowledge information database, and Wan fang databases. Retrieved articles were reviewed to select related data of our interest. References included in the literature were also searched and reviewed to find other potentially eligible data.

**Inclusion criteria**

The studies included in the meta-analysis must meet the following three criteria: (1) evaluating the association between ACE rs4646994 polymorphisms and HCM / DCM; (2) a case-control design was used, and (3) the data had to include the genotypes of II, ID, and DD, as well as comprehensive statistical indexes that were direct or indirect: odd ratio (or) and 95% confidence interval (95% CI) and fulfilled the hardy Weinberg equilibrium (HWE) among the control group.
Exclusion criteria

All patients were excluded for potential influencing factors such as hypertension, hypertensive heart disease, coronary atherosclerotic heart disease, ischemic heart disease, ischemic cardiomyopathy, severe coronary obstruction for DCM, valvular heart disease, valvular heart disease, congenital heart or vascular malformations, and inherent pulmonary disease.

Data extraction

Two authors independently reviewed all included studies and extracted vital data. Disagreements were resolved by a third researcher, and a common outcome was finally reached. We extracted the following information: first author, year of publication, country from which subjects came, ethnicity, number of cases and controls, allele and genotype frequencies, source of control group, diagnostic criteria and HWE test. We have attempted to contact the original authors if study data are incomplete. Study quality was assessed by the Newcastle Ottawa Scale (NOS).

Statistical methods

HWE was performed in the control group, and the significance level was set at P < 0.05. The association between ACE rs4646994 polymorphisms and HCM / DCM was assessed by fixed or random effects models incorporating ORs and 95% CIs. We demonstrated the degree of heterogeneity between studies by using $I^2$ ranging from 0% (complete agreement) to 100% (complete inconsistency). We used a random effects model (Der Simonian and Laird method) for the pooled analysis, and $I^2 > 50\%$ indicated heterogeneity among studies. Otherwise, a fixed effects model (mantel Haenszel method) should be used. We also performed subgroup analysis to identify possible heterogeneity and cumulative meta-analysis to determine the reliability of the results. All analyses were performed in 5 genetic models: allelic model (d vs. I), homozygous
model (DD vs. II), heterozygous model (ID vs. II), dominant model (ID + DD vs. II) and recessive model (DD vs. ID + II). Sensitivity analyses assessed the potential impact of individual study datasets on pooling or omitting studies. We also performed egger's test and plotted Begg's funnel plot to determine publication bias, and concluded that there was no statistically significant publication bias when p > 0.05. All statistical tests were performed using Stata version 15.0 (Stata Corp, University of Texas).

**Results**

**Research characteristics**

Finally we found a total of 368 potential articles related to keywords, of which 48 duplicate studies were excluded. We then initially screened the remaining 320 articles, 263 of which were excluded. From the full-text reading of 57 articles, 28 were excluded because of their disassociation with ACE rs4646994 polymorphisms and HCM / DCM (n = 10), review (n = 9), insufficient data (n = 7) and deviation from the HWE test (n = 2). The entire process of exclusion and enrollment is shown in Figure 1. Finally, this meta-analysis included 13 studies on DCM (2004 controls and 1376 cases, Table 1) and 16 studies on HCM (2161 controls and 1192 patients, Table 2). The NOS scores of each study were more than 6, and the quality was good. The results are shown in Table 1 and Table 2.

**Association between ACE rs4646994 polymorphism and susceptibility to DCM**

Our meta-analysis showed that potential heterogeneity was found in all five genetic models (allele model: \( I^2: 69.6\% \); homozygous gene model: \( I^2: 71.7\% \); heterozygous gene model: \( I^2: 74.3\% \); dominant gene model: \( I^2: 74\% \); recessive gene model: \( I^2: 64.3\% \)). Therefore, a random-effects model is used in the meta-analysis (Figure 2). The results of the study on the association between
ACE rs4646994 polymorphism and the pathogenesis of DCM showed that allele gene model (D versus I): OR=1.39, 95%CI=1.14-1.69, P=0.001; homozygote gene model (DD versus II): OR=2.02, 95%CI=1.32-3.09, P=0.001; heterozygote gene model (ID versus II): OR=1.46, 95%CI=1.01-2.12, P=0.045; dominance gene model (ID+DD vs II): OR=1.62, 95%CI=1.14-2.29, P=0.006; recessive gene model (DD vs ID and II): OR=1.53, 95%CI=1.12-2.08, P=0.007. In summary, our meta-analysis showed that there was a significant association between ACE rs4646994 polymorphism and DCM in the five gene models. It can be concluded that the D allele and DD genotype of ACE rs4646994 polymorphism may be the genetic risk factors of DCM.

We try to determine more reliable results and explore the sources of heterogeneity by analyzing different subgroups. First of all, a subgroup analysis is carried out on the ethnicity (Asian race and White race). As shown in Table 3, the results show that four gene models of Asian race suggest that there is a significant association between ACE rs4646994 polymorphism and DCM (allele gene model: OR=1.47, 95%CI=1.21-1.78, P<0.001; homozygous gene model: OR=2.28, 95%CI=1.49-3.47, P<0.001; dominant gene model: OR=1.72, 95%CI=1.12-2.64, P=0.01; recessive gene model: OR=1.67, 95%CI=1.16-2.39, P=0.05). However, no association was shown between the Asian heterozygous gene model (heterozygous gene model: OR=1.51, 95%CI=0.91-2.50, P=0.11) and the white subgroup (allele gene model: OR=1.25, 95%CI=0.85-1.84, P=0.27; homozygous gene model: OR=1.61, 95%CI=0.70-3.67, P=0.26; Heterozygous gene model: OR=1.27, 95%CI=0.77-2.10, P=0.35; dominant gene model: OR=1.40, 95%CI=0.78-2.51, P=0.26; recessive gene model: OR=1.29, 95%CI=0.74-2.24, P=0.37). The results showed that the mutation of ACE gene significantly increased the risk of DCM in Asian population. Although there was no
statistical significance between the mutation of ACE gene and the incidence of DCM in white population, it had a tendency to increase the risk of DCM. We conducted a subgroup analysis of the sample size, and the subgroup analysis of the sample size ≥ 200 showed that there was an association between ACE rs4646994 polymorphism and the DCM risk of the three gene models (allele gene model: OR=1.35, 95% CI=1.07-1.70, P=0.01; homozygous gene model: OR=1.97, 95% CI=1.17-3.30, P=0.01; recessive gene model: OR=1.47, 95% CI=1.04-2.08, P=0.03).

In the subgroup with sample size ≤ 200, this relationship disappeared (allele gene model: OR=1.49, 95% CI=1.00-2.23, P=0.05; homozygous gene model: OR=2.16, 95% CI=0.95-4.90, P=0.06; heterozygous gene model: OR=1.40, 95% CI=0.98-2.00, P=0.07; recessive gene model: OR=1.66, 95% CI=0.81-3.40, P=0.16).

In order to further determine the reliability of the results, through cumulative meta-analysis, we find that the more stable the association between ACE rs4646994 polymorphism and the incidence of DCM is as the year of publication approaches (Figure 3). It indicates that the results of this meta-analysis are very reliable.

**Association between ACE rs4646994 polymorphism and susceptibility to HCM**

Our meta-analysis showed that there was a significant association between ACE rs4646994 polymorphism and HCM in four genetic models: allele gene model (D vs I): OR=1.36, 95% CI=1.13-1.63, P=0.001; homozygous gene model (DD vs II): OR=1.80, 95% CI=1.21-2.67, P=0.003; heterozygous gene model (ID vs II): OR=1.76, 95% CI=1.29-2.40, P<0.001; dominant gene model (ID+DD vs II): OR=1.77, 95% CI=1.30-2.41, P<0.001. The difference is that the recessive gene model (DD vs ID and II: OR=1.28, 95% CI=0.99-1.67, P=0.064) shows that ACE gene mutation has nothing to do
with HCM. However, the trend of increasing risk can still be seen. The results of the Forest plot are shown in Figure 4.

In order to determine more reliable results and explore the source of heterogeneity, we conducted a subgroup analysis. First of all, we conducted a subgroup analysis of ethnicity, and table 4 showed that the mutation of ACE gene was not associated with the incidence of HCM in White population (allele gene model: OR=1.19, 95%CI=0.91-1.54, P=0.02; homozygous gene model: OR=1.40, 95%CI=0.83-2.35, P=0.212; heterozygous gene model: OR=1.18, 95%CI=0.81-1.74, P=0.39; dominant gene model: OR=1.25, 95%CI=0.82-1.91, P=0.29; recessive gene model: OR=1.21, 95%CI=0.87-1.68, P=0.26).

Although the recessive gene model (OR=1.31, 95%CI=0.87-1.97, P=0.20) analysis in Asian population showed that there was no association between ACE rs4646994 polymorphism and the incidence of HCM, there was a significant association among the other four models (allele gene model: OR=1.49, 95%CI=1.20-1.85, P<0.001; homozygous gene model: OR=2.06, 95%CI=1.10-3.87, P=0.02; dominant gene model: OR=1.89, 95%CI=1.06-3.36, P=0.03), while the other two showed nothing to do with
it (heterozygous gene model: OR = 1.78, 95% CI = 1.00-3.15, P = 0.05; recessive gene model: OR = 1.36, 95% CI = 0.95-1.94, P = 0.10). Similarly, the results in the subgroup with sample size ≤ 200 also showed that there was an association between the three gene models (allele gene model: OR = 1.33, 95% CI = 1.06-1.68, P = 0.02; heterozygous gene model: OR = 1.71, 95% CI = 1.27-2.30, P < 0.001; dominant gene model: OR = 1.71, 95% CI = 1.27-2.30, P < 0.001), while there was no such phenomenon in the other two gene models (homozygous gene model: OR = 1.61, 95% CI = 0.99-2.64, P = 0.06; recessive gene model: OR = 1.18, 95% CI = 0.78-1.79, P = 0.44).

As shown in Figure 5, through cumulative meta-analysis, we find that with the passage of time of the five gene models, the more stable the association between ACE rs4646994 polymorphism and the risk factors of HCM is.

Sensitivity analysis

We conducted the sensitivity analysis to assess whether omitting each study would change the overall ORs. As shown in Supplementary Figure 1 and 2, none of the studies would change the results of our meta-analysis, which showed that our results are reliable.

Publication bias

The Begg’s funnel plots associated with the above analyses are presented in Supplementary Figure 3 and 4. From the Begg’s funnel plot, it can be seen that there was no obvious asymmetry in each meta-analysis, thus indicating that there was no publication bias in our study. We performed Egger’s test to further validate the above conclusion (DCM: allele model: P = 0.068; homozygote model: P = 0.051; heterozygote model: P = 0.121; dominant model: P = 0.040; and recessive model: P = 0.079. HCM: allele model: P = 0.472; homozygote model: P = 0.678; heterozygote model: P = 0.343; dominant model: P = 0.087; and recessive model: P = 0.897).
Discussion

In this meta-analysis, we critically reviewed all eligible published studies that met the inclusion and exclusion criteria to evaluate the association between ACE rs4646994 polymorphisms and the risk of DCM/HCM. There were thirteen studies regarding DCM and sixteen on HCM. Our findings suggest that ACE rs4646994 polymorphisms may be associated with both HCM and DCM.

Polymorphisms in the ACE gene, encoding one of the components of the renin angiotensin aldosterone system (RAAS), have been found to be associated with a variety of cardiovascular diseases, such as hypertension, myocardial infarction, and cardiomyopathy. Increased synthesis of angiotensin II induces cell proliferation, migration, and hypertrophy and can enhance proinflammatory cytokine and matrix metalloproteinase production. Studies have shown that the anatomical features of HCM are characterized by asymmetric hypertrophy of the ventricles, whereas DCM is a class of cardiomyopathies characterized by systolic dysfunction of the left ventricle or biventricular enlargement class. Meanwhile, aldosterone production is regulated by the renin-angiotensin system, and studies have shown that it has a direct effect on the heart, including recurrent cardiac hypertrophy and fibrosis, ultimately leading to cardiac remodeling.

Therefore, ACE rs4646994 polymorphisms provisionally play an important role in the pathogenesis of HCM/DCM cardiomyopathy. While the results of our meta-analysis revealed that ACE rs4646994 polymorphism was associated with the risk of DCM/HCM incidence, providing a rationale of genetic aspects for the treatment of DCM/HCM.

Our subgroup analysis showed that ACE gene mutations can increase the risk of DCM and HCM in Asian population, while no such results were obtained for Caucasian population. The above
results suggest an association between the risk of incident DCM and HCM and the race. In addition, the population may be the source of heterogeneity because the heterogeneity was reduced in the subgroup analysis of the population. In the subgroup analysis with a dividing line of sample size 200, we found that the association of ACE gene mutations with the risk of DCM incidence was shown in the subgroup analysis with a sample size greater than 200 in DCM but not in the subgroup analysis with less than 200. Therefore, we believe that a larger sample size is needed for the association of DCM with ACE gene mutations to confirm the reliability of the results. Whereas in HCM the analysis of a subsample size, both showed an association of ACE gene mutations with the onset of HCM. The results of our time-series analyses all showed a stable relationship between ACE gene mutations and the risk of incident DCM/HCM. None of the studies could change the meta-analysis results in the sensitivity analysis (Supplementary Figure 1 and 2). No publication bias was found in our study (Supplementary Figure 3 and 4).

There are certain limitations to our study. First, we failed to group familial DCM/HCM and sporadic DCM/HCM due to limited data. Second, most of our reference studies had small sample sizes, which may affect the results of the meta-analysis. Third, the ethnic distribution of our included studies was relatively single, only Caucasian and Asian ethnicities were included, and subgroup analysis could not be performed for all ethnic populations. Besides, heterogeneity due to differences in the regression models of the included studies could not be avoided due to unavailability of specific information. The review protocol of this study was not pre-registered with PROSPERO.

**Conclusion**

This meta-analysis showed an association between the onset of HCM/DCM and ACE rs4646994
polymorphism. The findings of the current study may contribute to stratification strategies for patients with HCM/DCM. In addition, these results also show the potential possibility to treat HCM/DCM by modulating the RAAS system function in patients.

Data Availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing Interests

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

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**Author Contribution**

Jinsheng Shen and Yufeng Jiang contributed equally to this work. Jinsheng Shen and Yafeng Zhou designed the study. Jinsheng Shen, Yufeng Jiang, Yafeng Zhou, Xiaofei Mei, Jialu Yao, Hezi Jiang, Kexin Li and Tan Chen did the literature search, data extraction, statistical analysis, and drafted the figures. Jinsheng Shen wrote the first draft of the report, and Yafeng Zhou, Yufeng Jiang helped to write the final version. All authors read and met the criteria for authorship. All authors agree with the results and conclusions of the report.

**Ethics Approval and Consent to Participate**

Ethical approval was not needed because this is a meta-analysis.

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None

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Figure Legends

Figure 1: The PRISMA flow diagram of the study selection and exclusion. PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

Figure 2: Forest plot from the meta-analysis on the association of ACE rs4646994 gene polymorphism and DCM risk. A: allele; B: homozygote; C: heterozygote; D: dominant; and E: recessive.

Figure 3: The cumulative meta-analysis of the association of ACE rs4646994 gene polymorphism and DCM risk. A: allele; B: homozygote; C: heterozygote; D: dominant; and E: recessive.

Figure 4: Forest plot from the meta-analysis on the association of ACE rs4646994 gene polymorphism and HCM risk. A: allele; B: homozygote; C: heterozygote; D: dominant; and E: recessive.

Figure 5: The cumulative meta-analysis on the association of ACE rs4646994 gene polymorphism and HCM risk. A: allele; B: homozygote; C: heterozygote; D: dominant; and E: recessive.
Figure 1. The PRISMA flow diagram of the study selection and exclusion.
Table 1. The characteristics of included studies and ACE rs4646994 polymorphism genotype distribution and allele frequency of DCM in case group and control group.

| Author          | Year | Country | Ethnicity | sample size | Genotype (N) | Allele Frequency (N, %) | NOS score | HWE test |
|-----------------|------|---------|-----------|-------------|---------------|-------------------------|-----------|----------|
| Montgomery HE et al. | 1995 | UK      | Caucasian | 463         | II 18 ID 50 DD 31 | Total 84 168 112 364 | 86 112 0.57 336 | 392 0.54 | 6 0.173  |
| Sanderson JE et al. | 1996 | China   | Asian     | 200         | II 39 ID 49 DD 12 | Total 100 39 48 13 | 100 127 0.37 126 | 74 0.37 | 6 0.767  |
| Yamada Y et al. | 1997 | Japan   | Asian     | 210         | II 36 ID 35 DD 17 | Total 88 50 55 17 | 122 107 0.39 155 | 89 0.36 | 6 0.764  |
| Tiret L et al. | 2000 | Frech   | Caucasian | 809         | II 94 ID 200 DD 128 | Total 422 71 190 126 | 387 388 0.54 332 | 442 0.57 | 6 0.966  |
| Shan J et al. | 2001 | China   | Asian     | 238         | II 27 ID 25 DD 31 | Total 83 50 80 25 | 155 79 0.37 122 | 100 0.37 | 6 0.764  |
| Wu GR et al. | 2002 | China   | Asian     | 106         | II 14 ID 22 DD 7 | Total 43 23 28 12 | 63 50 0.42 74 | 52 0.41 | 6 0.509  |
| Zou DL et al. | 2005 | China   | Asian     | 96          | II 12 ID 18 DD 13 | Total 43 28 20 5 | 53 42 0.51 76 | 30 0.28 | 6 0.609  |
| Rai TS et al. | 2008 | India   | Asian     | 215         | II 8 ID 33 DD 10 | Total 51 47 87 30 | 164 49 0.52 181 | 147 0.45 | 6 0.353  |
| Kucukarabaci B et al. | 2008 | Turkey  | Caucasian | 49          | II 5 ID 18 DD 6 | Total 29 7 9 4 | 20 28 0.52 23 | 17 0.43 | 6 0.722  |
| Mahjoub S et al. | 2010 | Tunisia | Caucasian | 227         | II 12 ID 38 DD 26 | Total 76 46 83 22 | 151 62 0.59 175 | 127 0.42 | 6 0.116  |
| Kong YQ et al. | 2012 | China   | Asian     | 206         | II 20 ID 49 DD 32 | Total 101 30 53 22 | 105 89 0.56 113 | 97 0.46 | 6 0.874  |
| Rani B et al. | 2017 | India   | Asian     | 377         | II 15 ID 120 DD 42 | Total 177 72 86 42 | 200 150 204 0.58 | 230 170 0.43 | 6 0.089  |
| Chen W et al. | 2017 | China   | Asian     | 184         | II 17 ID 29 DD 18 | Total 64 51 57 12 | 120 63 0.51 159 | 81 0.34 | 6 0.496  |

N: number; NOS: Newcastle-Ottawa Scale; HWE: Hardy-Weinberg equilibrium; RAF: Risk Allele Frequency; risk allele: D allele; I: wild type; D: mutant type.
Table 2. The characteristics of included studies and ACE rs4646994 polymorphism genotype distribution and allele frequency of HCM in case group and control group.

| Author         | Year | Country | Ethnicity | Sample size | Genotype (N) | Allele Frequency (N, %) | NOS score | HWE test |
|----------------|------|---------|-----------|-------------|---------------|------------------------|-----------|----------|
| Marian AJ et al. | 1993 | USA     | Caucasian | 206         | II 7, ID 49, DD 44 | Total 100, I 22, D 46, DD 38 | 106, I 63, D 137, DD 0.69 | 90, I 90, D 122, DD 0.58 | 6, 0.778 |
| Yamada Y et al.  | 1997 | Japan   | Asian     | 193         | ID 31, II 32, D 8 | Total 71, ID 50, D 48 | 172, ID 48, D 0.34, DD 0.48 | 155, ID 89, D 0.36 | 6, 0.667 |
| Moiseev VS et al. | 1997 | Russia  | Caucasian | 181         | II 2, ID 5, DD 6 | Total 13, II 13, ID 33, DD 55 | 80, II 9, ID 17, D 0.65 | 121, II 121, ID 0.64 | 6, 0.315 |
| Lopez-Haldon J   | 1999 | Spain   | Caucasian | 309         | II 2, ID 13, DD 25 | Total 40, II 132, ID 33, DD 111 | 269, II 17, ID 63, D 0.79 | 191, II 191, ID 0.64 | 6, 0.952 |
| Cai et al.       | 2000 | China   | Asian     | 101         | II 16, ID 16, DD 13 | Total 45, II 26, ID 23, DD 7 | 56, II 48, ID 42, D 0.47 | 75, II 75, ID 37, DD 0.33 | 6, 0.528 |
| Gao et al.       | 2000 | China   | Asian     | 101         | II 12, ID 15, DD 13 | Total 40, II 31, ID 18, DD 12 | 61, II 39, ID 41, D 0.51 | 80, II 80, ID 42, DD 0.34 | 6, 0.185 |
| Yang et al.      | 2000 | China   | Asian     | 149         | II 13, ID 35, DD 15 | Total 63, II 37, ID 36, DD 13 | 86, II 61, ID 65, D 0.52 | 110, II 110, ID 62, DD 0.36 | 6, 0.86 |
| Li et al.        | 2001 | China   | Asian     | 96          | II 13, ID 19, DD 1 | Total 33, II 28, ID 23, DD 12 | 63, II 45, ID 42, D 0.32 | 79, II 79, ID 47, DD 0.37 | 6, 0.001 |
| Ogiimoto A et al. | 2002 | Turkey  | Caucasian | 343         | II 53, ID 64, DD 21 | Total 138, II 83, ID 95, DD 27 | 205, II 170, ID 106, D 0.38 | 261, II 261, ID 149, DD 0.36 | 6, 0.653 |
| Zou et al.       | 2003 | China   | Asian     | 66          | II 5, ID 7, DD 1 | Total 13, II 28, ID 20, DD 5 | 53, II 17, ID 9, D 0.35 | 76, II 76, ID 30, DD 0.28 | 6, 0.052 |
| Kawaguchi H et al. | 2003 | Japan   | Asian     | 168         | II 26, ID 41, DD 13 | Total 80, II 43, ID 28, DD 17 | 88, II 93, ID 67, D 0.42 | 114, II 114, ID 62, DD 0.35 | 6, 0.661 |
| Doolan G et al.  | 2004 | Australia | Caucasian | 236         | II 10, ID 14, DD 12 | Total 36, II 48, ID 94, DD 58 | 200, II 34, ID 38, D 0.53 | 190, II 190, ID 210, DD 0.52 | 6, 0.147 |
| Rai TS et al.    | 2008 | India   | Asian     | 282         | II 11, ID 63, DD 44 | Total 118, II 47, ID 87, DD 30 | 164, II 85, ID 151, D 0.64 | 181, II 181, ID 147, DD 0.45 | 6, 0.048 |
| Kaya CT et al.   | 2010 | Turkey  | Asian     | 83          | II 8, ID 34, DD 21 | Total 63, II 5, ID 9, DD 6 | 20, II 50, ID 76, D 0.6 | 20, II 20, ID 19, DD 0.52 | 6, 0.661 |
| Coto E et al.    | 2010 | Spain   | Caucasian | 507         | II 35, ID 100, DD 72 | Total 207, II 46, ID 135, DD 119 | 300, II 170, ID 244, D 0.59 | 227, II 227, ID 373, DD 0.62 | 6, 0.147 |
| Rani B et al.    | 2017 | India   | Asian     | 332         | II 16, ID 89, DD 27 | Total 132, II 72, ID 86, DD 42 | 200, II 121, ID 143, D 0.54 | 230, II 230, ID 170, DD 0.43 | 6, 0.048 |

N: number; NOS: Newcastle-Ottawa Scale; HWE: Hardy-Weinberg equilibrium; RAF: Risk Allele Frequency; risk allele: D allele; I: wild type; D: mutant type.
| Number of studies | Allele comparison D versus I | Homozygous DD versus II | Heterozygous ID versus II | Dominant ID + DD versus II | Recessive DD versus ID + II |
|------------------|-----------------------------|------------------------|--------------------------|----------------------------|-----------------------------|
|                  | OR  | 95% CI | P   | I² (%) | OR  | 95% CI | P   | I² (%) | OR  | 95% CI | P   | I² (%) | OR  | 95% CI | P   | I² (%) |
| Total            | 13  |         |     |        |     |         |     |        |     |         |     |        |     |         |     |        |
| Ethnicity        |     |         |     |        |     |         |     |        |     |         |     |        |     |         |     |        |
| Asian            | 9   | 1.47    | 1.21-1.78 | <0.001 | 48.5 | 0.05    | 2.28 | 1.49-3.47 | <0.001 | 51.1 | 0.04    | 1.51 | 0.91-2.50 | 0.11 | 78.2 | <0.001 | 1.72 | 1.12-2.64 | 0.01 | 73.7 | <0.001 | 1.67 | 1.12-2.39 | 0.05 | 52.2 | 0.03 |
| Caucasian        | 4   | 1.25    | 0.85-1.84 | 0.27 | 78.4 | 0.003   | 1.61 | 0.70-3.67 | 0.26 | 79.4 | 0.002  | 1.27 | 0.77-2.10 | 0.35 | 55.7 | 0.08   | 1.4  | 0.87-2.51 | 0.26 | 70.4 | 0.018  | 1.29 | 0.74-2.24 | 0.37 | 73  | 0.01 |
| Sample size      |     |         |     |        |     |         |     |        |     |         |     |        |     |         |     |        |
| > 200            | 8   | 1.35    | 1.07-1.70 | 0.01 | 74 | <0.001 | 1.97 | 1.17-3.30 | 0.01 | 77.2 | <0.001 | 1.43 | 0.84-2.46 | 0.19 | 83.9 | <0.001 | 1.6  | 0.99-2.59 | 0.06 | 82.4 | <0.001 | 1.47 | 1.04-2.08 | 0.03 | 68.4 | 0.02 |
| ≤ 200            | 5   | 1.49    | 1.00-2.23 | 0.05 | 64.5 | 0.02    | 2.16 | 0.95-4.90 | 0.06 | 62 | <0.001  | 1.4  | 0.98-2.00 | 0.07 | 0 | 0.584  | 1.62 | 1.06-2.49 | 0.03 | 32.8 | <0.001 | 1.66 | 0.81-3.40 | 0.16 | 60  | 0.04 |

I/D: insertion/deletion.
### Table 4: Subgroup analysis of association between ACE I/D gene polymorphism and HCM

| Number of studies | Allele comparison D versus I | Homozygous DD versus II | Heterozygous ID versus II | Dominant ID + DD versus II | Recessive DD versus ID + II |
|-------------------|-----------------------------|-------------------------|--------------------------|---------------------------|----------------------------|
|                   | OR 95% CI                     | OR 95% CI               | OR 95% CI                | OR 95% CI                 | OR 95% CI                  |
|                   | P I2 (%)                      | P I2 (%)                | P I2 (%)                 | P I2 (%)                  | P I2 (%)                   |
| Total             | 16                           |                         |                          |                           |                           |
| Ethnicity         |                              |                         |                          |                           |                           |
| Asian             | 10                           | 1.49 1.20-1.85          | 0.001 47 0.05 2.09 1.25-3.50 0.005 55.3 0.02 2.15 1.51-3.06 0.10 45.3 0.06 2.11 1.48-3.00 0.10 | 1.31 1.04-1.97 0.03 51.7 0.03 1.87-3.49 0.14 | 51.4 0.03 1.87-3.49 0.14 |
| Caucasian         | 6                             | 1.19 0.91-1.54          | 0.20 55.3 0.05 1.4 0.83-2.35 0.212 49.8 0.08 1.18 0.81-1.74 0.39 28.3 0.22 1.25 0.82-1.91 0.29 44.3 0.11 1.21 0.87-1.68 0.26 | 40.3 0.14 1.21 0.87-1.68 0.26 | |
| Sample size       |                               |                         |                          |                           |                           |
| > 200             | 7                             | 1.39 1.05-1.84          | 0.02 76.4 0.001 2.06 1.10-3.87 0.02 76.9 0.001 1.78 1.00-3.15 0.05 77.8 0.001 1.89 1.06-3.36 0.03 80.3 0.001 1.36 0.95-1.94 0.10 | 64.9 0.04 1.36 0.95-1.94 0.10 | |
| ≤ 200             | 9                             | 1.33 1.06-1.68          | 0.02 29.5 0.18 1.61 0.99-2.64 0.06 30.7 0.17 1.71 1.27-2.30 0.001 0 0.50 1.65 1.25-2.19 0.001 2.8 0.41 1.18 0.78-1.79 0.44 | 28.3 0.19 1.18 0.78-1.79 0.44 | |

I/D: insertion/deletion.
Title: Effect of rs4646994 polymorphism of angiotensin converting enzyme on the risk of nonischemic cardiomyopathy

Running head: ACE rs4646994 polymorphism and nonischemic cardiomyopathy

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Supplementary Materials

Supplementary figure 1: Sensitivity analysis of the association of ACE rs4646994 gene polymorphism and DCM risk. A: allele; B: homozygote; C: heterozygote; D: dominant; and E: recessive.

Supplementary figure 2: Sensitivity analysis of the association of ACE rs4646994 gene polymorphism and HCM risk. A: allele; B: homozygote; C: heterozygote; D: dominant; and E: recessive.

Supplementary figure 3: Begg funnel plot with pseudo 95% confidence limits of the association of ACE rs4646994 gene polymorphism and DCM risk. A: allele; B: homozygote; C: heterozygote; D: dominant; and E: recessive.

Supplementary figure 4: Begg funnel plot with pseudo 95% confidence limits of the association of ACE rs4646994 gene polymorphism and HCM risk. A: allele; B: homozygote; C: heterozygote; D: dominant; and E: recessive.
