Association of angiotensinogen gene *M235T* and angiotensin-converting enzyme gene *I/D* polymorphisms with essential hypertension in Han Chinese population: a meta-analysis

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Background The polymorphisms of angiotensinogen (*AGT*) and angiotensin-converting enzyme (*ACE*) genes have been linked to increased risk of essential hypertension in multiple populations, but the results were inconsistent.

Objectives and methods To evaluate the associations of these polymorphisms with essential hypertension, we carried out a meta-analysis of the association studies within Han Chinese population. In this study, we reviewed two most commonly investigated polymorphisms, *AGT M235T* and *ACE I/D*, and provided summary estimates regarding their associations with essential hypertension. *PubMed* and *China Biological Medicine Database* were searched, and a total of 71 studies (31 studies for *AGT M235T* and 40 studies for *ACE I/D*) comprising 10 547 essential hypertension patients and 9217 controls from 23 provinces and special districts in China were finally included in this study.

Results Statistically significant associations with essential hypertension were identified for TT genotype of *AGT M235T* polymorphism (odds ratio 1.54, 95% confidence interval 1.16–2.03, *P* = 0.002) and DD genotype of *ACE I/D* polymorphism (odds ratio 1.61, 95% confidence interval 1.32–1.98, *P* < 0.0001). Under dominant, recessive, and additive genetic models, positive associations were also found. The heterogeneity existed among the studies (*P* < 0.05), whereas the publication bias did not exist in both *AGT* analysis (*P* = 0.052) and *ACE* analysis (*P* = 0.103).

Conclusion The present meta-analysis suggests that *AGT M235T* and *ACE I/D* modulate the risk of essential hypertension in Han Chinese population. *J Hypertens* 28:419–428 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Essential hypertension, defined as high blood pressure (BP) without any obvious cause, constitutes about 95% of all hypertension cases. The cause of hypertension is considered polygenic, resulting from the interaction of several genes and together with environmental factors such as dietary sodium intake, excess alcohol consumption, obesity, and stress [1,2]. Candidate genes in recent genetic studies are mostly selected from physiological systems, which have been implicated in BP regulation, particularly the renin–angiotensin system (RAS) [3]. Tremendous effort has been directed towards the understanding of RAS and its role in the regulation of BP [4]. Hitherto, hundreds of polymorphisms within *RAS* genes have been reported to be significantly associated with essential hypertension [5]. Among them, *AGT* gene encoding angiotensinogen (AGT) and *ACE* gene encoding angiotensin-converting enzyme (ACE) were investigated most deeply for their critical importance in the regulation of BP (Fig. 1). Furthermore, among all the reported polymorphisms in the two genes, *AGT M235T* and *ACE I/D* polymorphisms are the most prominent loci.

Jeunemaitre *et al.* [6] first reported significant evidence for linkage and association of genetic variants in *AGT* gene (especially *M235T*, which results in the substitution of threonine for methionine at amino acid residue 235) with essential hypertension. Since then, the association of *AGT M235T* with essential hypertension has been successfully replicated in many independent populations and consequently became one of the most promising candidate loci [7–11]. Furthermore, Pereira *et al.* [12] also found a dose-dependent association between the T allele of this polymorphism and increased BP in a large, ethnically mixed urban population sample. Unfortunately, considerable opposite results were also published

Fig. 1
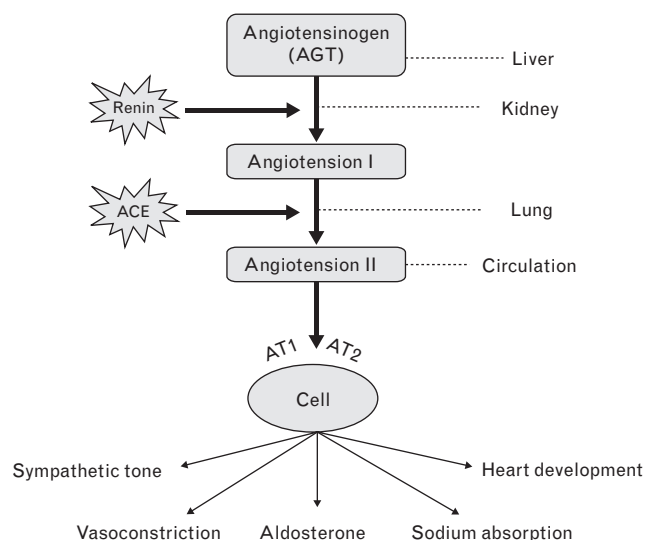
Schematic of renin−angiotensin system and its applications. Angiotensinogen is a liver protein that interacts with renin to produce angiotensin I, whereas ACE catalyzes the conversion of decapeptide angiotensin I to octapeptide angiotensin II, which is the major effector molecule of the RAS that plays a key role in regulation of blood pressure by increasing vascular tone and promoting sodium retention. ACE, angiotensin-converting enzyme; AGT, angiotensinogen; AT1/2, angiotensin I/II; RAS, renin−angiotensin system.

regarding the association of the *AGT M235T* polymorphism with essential hypertension [2,11].

The *ACE I/D* polymorphism identified in 1990 by Rigat *et al*. [13] is another polymorphism that has been extensively studied. This polymorphism in the *ACE* gene is detailed on the presence or absence of a 287-bp *Alu* element in intron 16 on chromosome 17. Rigat *et al*. [13] have shown that the level of circulating ACE depends on this insertion/deletion (*I/D*) polymorphism. Subsequently, some other studies also demonstrated a strong correlation between the *D* allele and the circulating as well as intralymphocytic levels of ACE [14,15]. Some researchers [16,17] also reported that the ACE activity was highest in *DD* homozygote and intermediate in *ID* heterozygote when compared with the *II* homozygote. However, like the situation for *AGT M235T*, the published results for this polymorphism were also conflicting [2,18−20].

In China, genetic association studies are also widely carried out to evaluate the relationship between these two polymorphisms and essential hypertension. Like the same situation in the worldwide populations, the obtained conclusions in Chinese populations are also inconsistent. Many reasons can be attributed to, among which, population structure as well as ethnic specificity are probably the most potential confounding factors. A meta-analysis of the association studies, which were conducted in a genetically well defined population, could resolve part of the controversy. Therefore, we carried out a meta-analysis for these two polymorphisms in Han Chinese population by analyzing the pooled estimates of the associations between the *AGT M235T* as well as *ACE I/D* polymorphisms and essential hypertension.

Methods
Literature search
According to the Egger *et al*.'s [21] literature searching approach, studies addressing the relationship between the *AGT M235T* as well as *ACE I/D* polymorphisms and essential hypertension were found by two investigators who independently searched the *PubMed* (http://www.ncbi.nlm.nih.gov/pubmed/) and *China Biological Medicine Database* (*CBM*, http://cbm.imicams.ac.cn/). *CBM* is the biggest database of Chinese biomedical research literature, a similar Chinese version of *MEDLINE*. We search the articles within a range of published years from 1996 to 2008, and the article language is limited to Chinese or English. The terms used for searching in 'MeSH terms' were 'hypertension', 'angiotensin-converting enzyme', 'angiotensinogen', 'polymorphism', 'genotype', 'gene', 'allele', and 'mutation', in combination with 'Han' in all fields of the article.

Selection criteria
The data were included in the following meta-analysis if the research papers fulfilled the subsequent two conditions: the association of essential hypertension and *AGT M235T* or *ACE I/D* polymorphism were examined in Han Chinese individuals; the genotype frequencies were reported in both patients and controls. After that, we re-evaluated the Hardy−Weinberg equilibrium (HWE) for every included study result, and the studies that did not follow HWE were excluded. We also carefully examined the publications for possible overlap in the recruitment of the participants. Individuals enrolled in more than one article on the same outcome variable were counted only once in the pooled statistics.

Data extraction
In addition to the retrieval criteria mentioned above, we collected the following information from each study: author, year of publication, and region; number of genotyped participants; and distribution of genotypes and alleles in both control and hypertension groups. The citations were ordered by year of publication and their first author, given a unique identification number and entered into a dedicated literature database. All the extracted information was checked by other research personnel. First, one investigator read all papers, extracted and computerized the relevant information, and provided copies of the papers and the database to two collaborators. Both checked the extracted data in an independent manner. Inconsistencies were corrected or discussed until a unanimous interpretation of the source data was reached.

Statistical analysis methods

The odds ratio (OR) was used to compare the distributions of alleles and genotypes between patients and controls using the STATA software (STATA Corp., College Station, Texas, USA; version 10.0). Test for heterogeneity were performed for each meta-analysis with the I^2 test [22] (significance set at $P < 0.05$). Where there was heterogeneity in studies, a pooled OR was estimated by the random effects model (DerSimonian–Laird method) [23]; otherwise, the fixed effects model (Mantel–Haenszel method) [24] was used. The fixed effects model assumes homogeneity among study estimates and is used when there is no evidence for heterogeneity. Conversely, when heterogeneity is existed, a random effects model is usually more appropriate because it takes into account the between-study variability. Funnel plots were used to investigate publication bias. Linear regression approach was used to measure funnel plot asymmetry on the natural logarithm scale of the OR (Egger *et al.*'s test) [25]. Evidence of asymmetry was based at a *P* value of less than 0.05 statistical level.

Results

Description of data

For *AGT M235T*, the literature search generated 73 papers, of which 31 articles met the selection criteria. From the 42 excluded articles, four were duplicate publication, seven were reviews, nine did not follow HWE, and 22 studied the other endpoints or other polymorphisms.

For *ACE I/D*, the literature search generated 118 papers, of which 40 articles met the selection criteria. From the 78 excluded articles, 10 were duplicate publication, 12 were reviews, 14 did not follow HWE, 41 studied the other endpoints or other polymorphisms, and one was without distribution of genotypes.

A total of 71 studies (31 studies for *AGT M235T* and 40 studies for *ACE I/D*), comprising 10 547 essential hypertension patients and 9217 controls from 23 provinces and special districts in China (see Fig. 2 and supplementary material), fulfilled the criteria for inclusion in this study.

In the meta-analysis of *AGT M235T* polymorphism, the frequency of the T allele was 75.2% for hypertension group and 72.7% for control. In addition, the respective prevalence rates of TT/MT/MM for hypertension and control were 57.3 and 53.3%, 35.8 and 38.8%, and 6.9 and 7.9%, respectively.

In the meta-analysis of *ACE I/D* polymorphism, the frequency of the D allele was 46.0% for hypertension group and 41.2% for control. In addition, the respective

Fig. 2

Distribution of sample source in China. The participants were selected from 23 provinces and special districts in China.

prevalence rates of *DD/ID/II* genotypes for hypertension and control were 24.3 and 17.8%, 43.3 and 46.8%, and 32.3 and 35.4%, respectively. Among these studies, one study concluded that *II* was associated with hypertension, whereas the other positive studies suggested *DD* was associated with hypertension.

Broad analysis

For *AGT M235T* polymorphism, the summary OR for TT versus MM is shown in Fig. 3 and Table 1, and the summary OR was 1.54 [95% confidence interval (95% CI) 1.16–2.03] by random effects model ($P = 0.002$). The heterogeneity between the 31 study comparisons was significant ($P = 0.014$, $I^2 = 39.2\%$). The summary OR for MT versus MM is shown in Fig. 4 and Table 1, and the summary OR was 1.08 (95% CI 0.82–1.42) by random effects model ($P = 0.591$). The heterogeneity between the 31 study comparisons was significant ($P = 0.008$, $I^2 = 41.9\%$). As shown in Table 1, the summary OR for dominant model was 1.28 (95% CI

1.07–1.53) by random effects model ($P = 0.025$). The summary OR for recessive model was 1.51 (95% CI 1.20–1.91) by random effects model ($P = 0.001$). The summary OR for additive model was 1.38 (95% CI 1.17–1.62) by random effects model ($P < 0.0001$).

For *ACE I/D* polymorphism, the summary OR for *DD* versus *II* is shown in Fig. 5 and Table 2, the summary OR was 1.61 (95% CI 1.32–1.98) by random effects model ($P < 0.0001$). The heterogeneity between the 40 study

Table 1　Meta-analysis of association between *AGT M235T* polymorphism and essential hypertension risk

	Pooled OR (95% CI)	Z (P)	I^2 (%)
TT versus MM	1.54 (1.16–2.03)	3.03 (P = 0.002)	39.2
MT versus MM	1.08 (0.82–1.42)	0.54 (P = 0.591)	41.9
Dominant	1.28 (1.07–1.53)	2.25 (P = 0.025)	33.6
Recessive	1.51 (1.20–1.91)	3.47 (P = 0.001)	82.1
Additive	1.38 (1.17–1.62)	3.89 (P < 0.0001)	76.4

CI, confidence interval; OR, odds ratio.

Fig. 3

Name	Year	OR (95% CI)	% Weight
Chiang	1997	0.90 (0.00, .)	0.69
Cheung	1998	3.16 (0.44, .)	1.27
Gong	1998	5.73 (0.00, .)	0.79
Liu	1998	1.33 (0.48, 3.65)	4.01
Xiao	1998	8.01 (0.00, .)	0.75
Wang	1999	2.77 (0.90, 8.48)	3.52
Guan	2000	1.51 (0.53, 4.28)	3.90
Liu	2000	3.60 (1.20, 10.75)	3.64
Thomas	2000	1.39 (0.52, 3.67)	4.18
Li	2001	3.62 (0.50, .)	1.27
Wei	2001	2.76 (0.84, 9.09)	3.33
Sun	2002	3.84 (1.31, 11.14)	3.75
Wu	2002	2.19 (0.86, 5.61)	4.42
Yang	2002	0.90 (0.41, 2.02)	5.13
Kong	2003	0.80 (0.35, 1.83)	4.98
Lin	2003	5.53 (1.71, 17.71)	3.32
Liu	2003	1.42 (0.44, 4.57)	3.34
Wang	2003	1.36 (0.41, 4.45)	3.28
Zheng	2003	0.37 (1.78, 1.78)	2.08
Cai	2004	3.19 (1.21, 8.35)	4.26
Kong	2004	0.73 (0.34, 1.58)	5.32
Wu	2004	0.38 (0.15, 1.02)	4.19
Zhang	2005	1.41 (0.72, 2.78)	5.94
Dong	2006	0.42 (0.18, 0.95)	5.05
Liang	2006	4.18 (0.90, .)	2.12
Zhang-Q	2006	10.07 (0.00, .)	0.85
Zhang-S	2006	8.11 (0.00, .)	0.79
Xing	2007	1.19 (0.63, 2.25)	6.21
Yin	2007	0.34 (3.08, 3.08)	0.81
Wan	2008	2.48 (0.89, 6.91)	3.99
Zhao	2008	1.48 (0.41, 5.34)	2.86
Overall (I-squared = 39.2%, P = 0.014)		1.54 (1.16, 2.03)	100.00

Note: Weights are from random effects analysis

Forest plot of the association of the *AGT M235T* polymorphism with essential hypertension. Pooled OR compared the *T235T* genotype versus *M235M* genotype is shown under a random effects model. Studies are ordered by the publication year. CI, confidence interval; OR, odds ratio.

Fig. 4
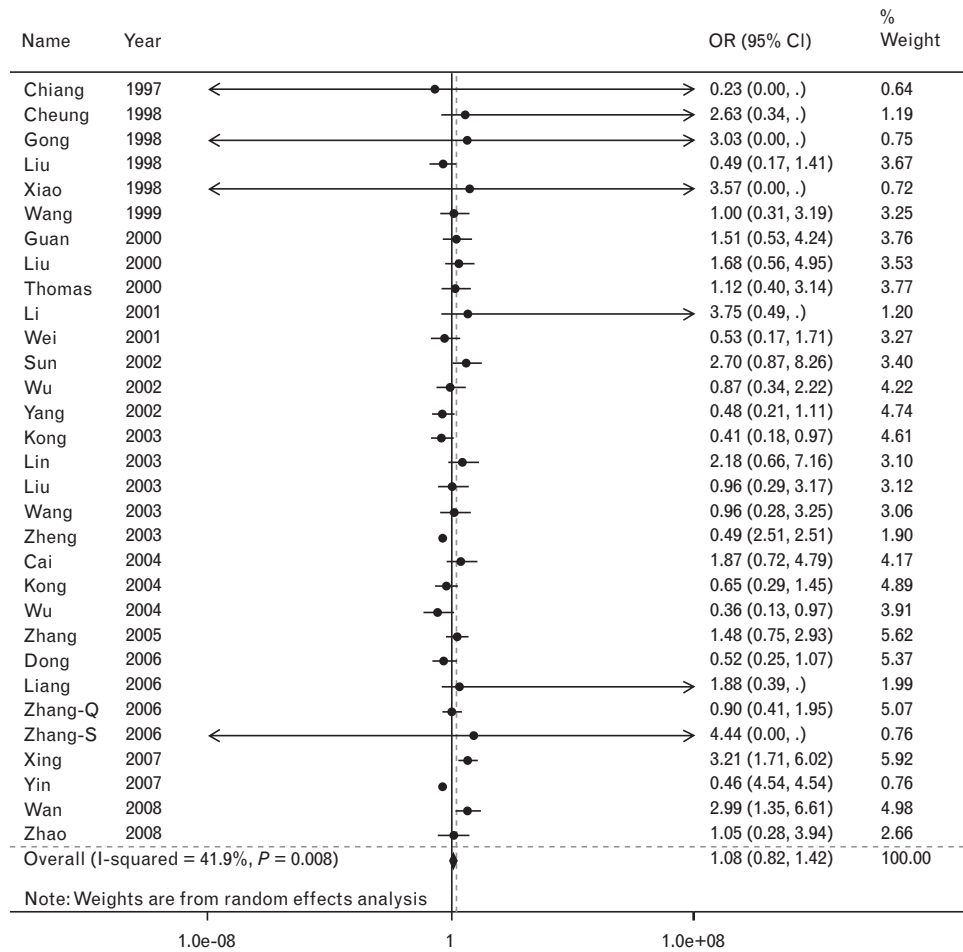
Forest plot of the association of the *AGT M235T* polymorphism with essential hypertension. Pooled OR compared the *M235T* genotype versus *M235M* genotype is shown under a random effects model. Studies are ordered by the publication year. CI, confidence interval; OR, odds ratio.

comparisons was significant ($P < 0.0001$, $I^2 = 68\%$). The summary OR for *ID* versus *II* is shown in Fig. 6 and Table 2, the summary OR was 1.00 (95% CI 0.89–1.14) by random effects model ($P = 0.963$). The heterogeneity between the 40 study comparisons was significant ($P = 0.001$, $I^2 = 44.8\%$). As shown in Table 2, the summary OR for dominant model was 1.18 (95% CI 1.03–1.35) by random effects model ($P = 0.018$). The summary OR for recessive model was 1.59 (95% CI 1.35–1.86) by random effects model ($P < 0.0001$). The summary OR for additive model was 1.27 (95% CI 1.14–1.42) by random effects model ($P < 0.0001$).
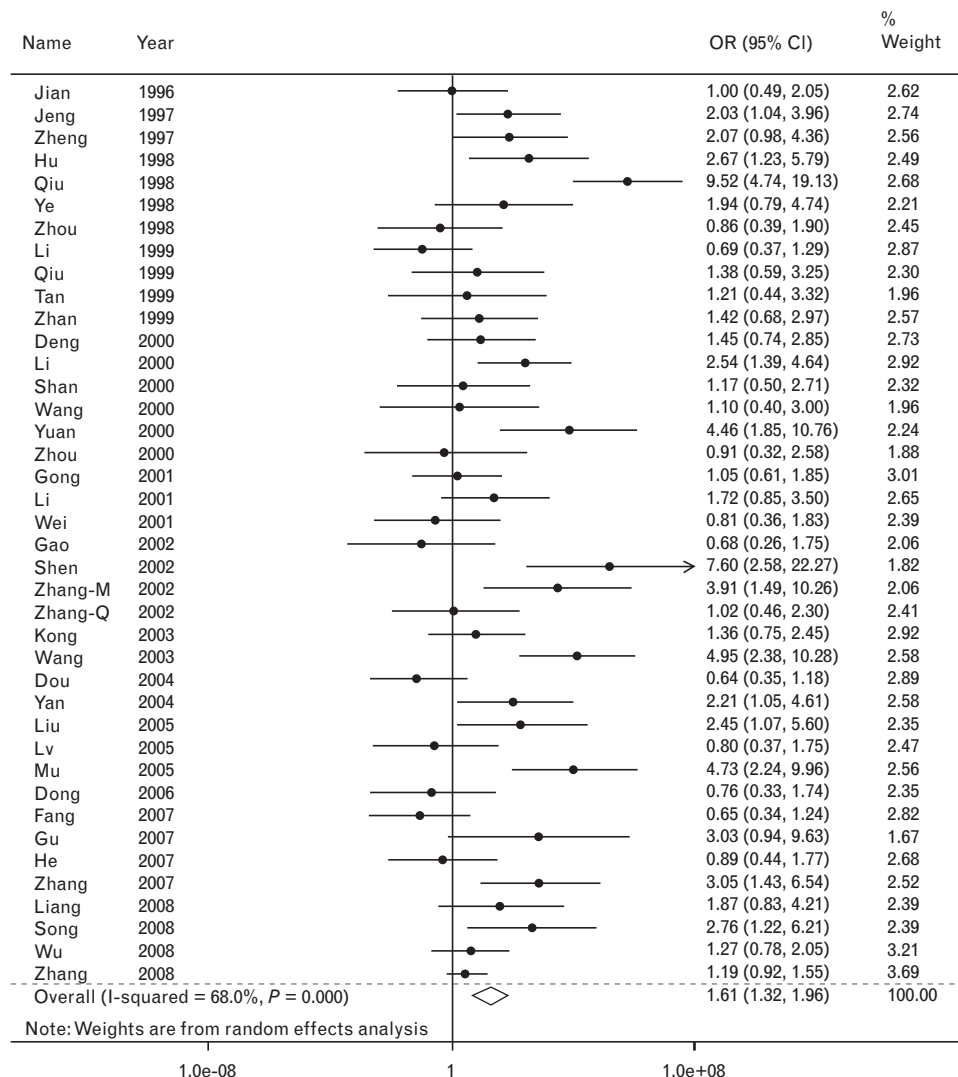
An important source of bias in every meta-analysis is publication bias. In our meta-analysis of *AGT M235T* and *ACE I/D* polymorphisms, there were many studies published with negative findings, so we found no evidence of publication bias by funnel plot visually (see Figs 7 and 8). Moreover, the Egger's test ($P = 0.052$ for *AGT* analysis

and $P = 0.103$ for *ACE* analysis) was not significant, suggesting a low probability of publication bias.

Discussion
Hypertension, defined as BP measurements of 140/90 mmHg or greater, has affected a quarter of the adult population in industrialized societies [26]. It is among major public health problems, which not only affect many developed countries but also some developing countries. In China, hypertension is one of the fastest growing diseases in the past 30 years. As mentioned by the Ministry of Health of the People's Republic of China (www.moh.gov.cn), there was an annual increase of one million patients with hypertension during the year 1950–1980. During 1981–1990, this number increased quickly to three million per year. In the past 15 years, the number kept on increasing significantly to seven million per year. Thus far, it is estimated that the prevalence of hypertension is approximately 18.8% in Chinese adult population.

Fig. 5

Name	Year	OR (95% CI)	% Weight
Jian	1996	1.00 (0.49, 2.05)	2.62
Jeng	1997	2.03 (1.04, 3.96)	2.74
Zheng	1997	2.07 (0.98, 4.36)	2.56
Hu	1998	2.67 (1.23, 5.79)	2.49
Qiu	1998	9.52 (4.74, 19.13)	2.68
Ye	1998	1.94 (0.79, 4.74)	2.21
Zhou	1998	0.86 (0.39, 1.90)	2.45
Li	1999	0.69 (0.37, 1.29)	2.87
Qiu	1999	1.38 (0.59, 3.25)	2.30
Tan	1999	1.21 (0.44, 3.32)	1.96
Zhan	1999	1.42 (0.68, 2.97)	2.57
Deng	2000	1.45 (0.74, 2.85)	2.73
Li	2000	2.54 (1.39, 4.64)	2.92
Shan	2000	1.17 (0.50, 2.71)	2.32
Wang	2000	1.10 (0.40, 3.00)	1.96
Yuan	2000	4.46 (1.85, 10.76)	2.24
Zhou	2000	0.91 (0.32, 2.58)	1.88
Gong	2001	1.05 (0.61, 1.85)	3.01
Li	2001	1.72 (0.85, 3.50)	2.65
Wei	2001	0.81 (0.36, 1.83)	2.39
Gao	2002	0.68 (0.26, 1.75)	2.06
Shen	2002	7.60 (2.58, 22.27)	1.82
Zhang-M	2002	3.91 (1.49, 10.26)	2.06
Zhang-Q	2002	1.02 (0.46, 2.30)	2.41
Kong	2003	1.36 (0.75, 2.45)	2.92
Wang	2003	4.95 (2.38, 10.28)	2.58
Dou	2004	0.64 (0.35, 1.18)	2.89
Yan	2004	2.21 (1.05, 4.61)	2.58
Liu	2005	2.45 (1.07, 5.60)	2.35
Lv	2005	0.80 (0.37, 1.75)	2.47
Mu	2005	4.73 (2.24, 9.96)	2.56
Dong	2006	0.76 (0.33, 1.74)	2.35
Fang	2007	0.65 (0.34, 1.24)	2.82
Gu	2007	3.03 (0.94, 9.63)	1.67
He	2007	0.89 (0.44, 1.77)	2.68
Zhang	2007	3.05 (1.43, 6.54)	2.52
Liang	2008	1.87 (0.83, 4.21)	2.39
Song	2008	2.76 (1.22, 6.21)	2.39
Wu	2008	1.27 (0.78, 2.05)	3.21
Zhang	2008	1.19 (0.92, 1.55)	3.69
Overall (I-squared = 68.0%, P = 0.000)		1.61 (1.32, 1.96)	100.00

Note: Weights are from random effects analysis

1.0e−08   1   1.0e+08

Forest plot of the association of the *ACE I/D* polymorphism with essential hypertension. Pooled OR compared the *DD* genotype versus *II* genotype is shown under a random effects model. Studies are ordered by the publication year. CI, confidence interval; OR, odds ratio.

This great proportion of hypertension patients in common society has exerted a large burden of medical expenditures. To better prepare for this severe situation, genetic studies for hypertension have been extensively carried out in China.
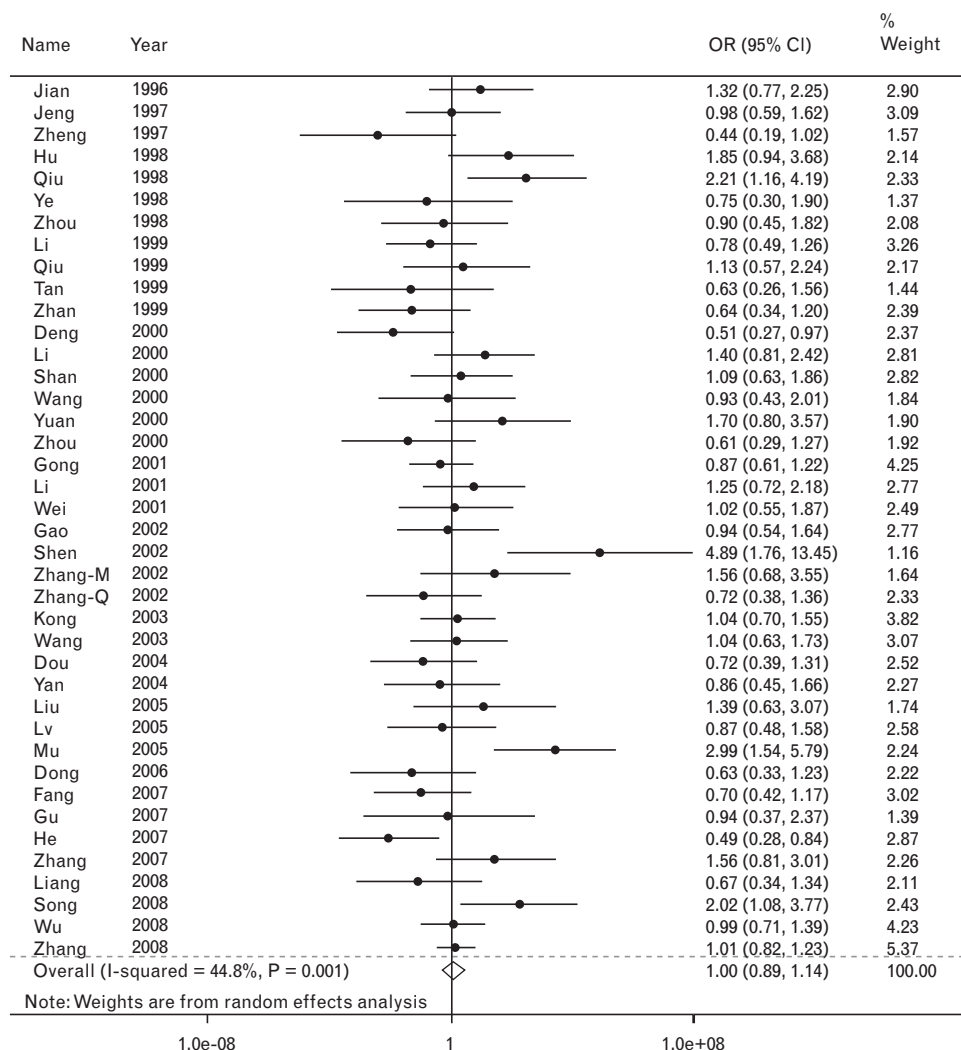
Table 2   Meta-analysis of association between *ACE I/D* polymorphism and essential hypertension risk

	Pooled OR (95% CI)	Z (P)	I^2 (%)
DD versus *II*	1.61 (1.32–1.98)	4.62 (P < 0.0001)	68.0
ID versus *II*	1.00 (0.89–1.14)	0.05 (P = 0.963)	44.8
Dominant	1.18 (1.03–1.35)	2.37 (P = 0.018)	60.4
Recessive	1.59 (1.35–1.86)	5.56 (P < 0.0001)	61.6
Additive	1.27 (1.14–1.42)	4.32 (P < 0.0001)	73.4

CI, confidence interval; OR, odds ratio.

Multiple polymorphisms have been reported to be associated with essential hypertension. Among them, *AGT M235T* and *ACE I/D* polymorphisms are the most extensively investigated ones. As conflicting findings for these two loci with essential hypertension had been reported, meta-analysis of the results reported from a well defined population may provide new information. Small effects in human genetic association studies can be detected by meta-analysis after pooled sample size and reduced chance of random error [27]. Therefore, published meta-analysis reports of genetic association studies are increasing quickly [2,16,28–31]. To arrive at a more robust and well powered estimate of the putative influence of *AGT M235T* and *ACE I/D* polymorphisms on essential hypertension, we carried out meta-analyses in

Fig. 6

Name	Year		OR (95% CI)	% Weight
Jian	1996		1.32 (0.77, 2.25)	2.90
Jeng	1997		0.98 (0.59, 1.62)	3.09
Zheng	1997		0.44 (0.19, 1.02)	1.57
Hu	1998		1.85 (0.94, 3.68)	2.14
Qiu	1998		2.21 (1.16, 4.19)	2.33
Ye	1998		0.75 (0.30, 1.90)	1.37
Zhou	1998		0.90 (0.45, 1.82)	2.08
Li	1999		0.78 (0.49, 1.26)	3.26
Qiu	1999		1.13 (0.57, 2.24)	2.17
Tan	1999		0.63 (0.26, 1.56)	1.44
Zhan	1999		0.64 (0.34, 1.20)	2.39
Deng	2000		0.51 (0.27, 0.97)	2.37
Li	2000		1.40 (0.81, 2.42)	2.81
Shan	2000		1.09 (0.63, 1.86)	2.82
Wang	2000		0.93 (0.43, 2.01)	1.84
Yuan	2000		1.70 (0.80, 3.57)	1.90
Zhou	2000		0.61 (0.29, 1.27)	1.92
Gong	2001		0.87 (0.61, 1.22)	4.25
Li	2001		1.25 (0.72, 2.18)	2.77
Wei	2001		1.02 (0.55, 1.87)	2.49
Gao	2002		0.94 (0.54, 1.64)	2.77
Shen	2002		4.89 (1.76, 13.45)	1.16
Zhang-M	2002		1.56 (0.68, 3.55)	1.64
Zhang-Q	2002		0.72 (0.38, 1.36)	2.33
Kong	2003		1.04 (0.70, 1.55)	3.82
Wang	2003		1.04 (0.63, 1.73)	3.07
Dou	2004		0.72 (0.39, 1.31)	2.52
Yan	2004		0.86 (0.45, 1.66)	2.27
Liu	2005		1.39 (0.63, 3.07)	1.74
Lv	2005		0.87 (0.48, 1.58)	2.58
Mu	2005		2.99 (1.54, 5.79)	2.24
Dong	2006		0.63 (0.33, 1.23)	2.22
Fang	2007		0.70 (0.42, 1.17)	3.02
Gu	2007		0.94 (0.37, 2.37)	1.39
He	2007		0.49 (0.28, 0.84)	2.87
Zhang	2007		1.56 (0.81, 3.01)	2.26
Liang	2008		0.67 (0.34, 1.34)	2.11
Song	2008		2.02 (1.08, 3.77)	2.43
Wu	2008		0.99 (0.71, 1.39)	4.23
Zhang	2008		1.01 (0.82, 1.23)	5.37
Overall (I-squared = 44.8%, P = 0.001)			1.00 (0.89, 1.14)	100.00

Note: Weights are from random effects analysis

1.0e-08   1   1.0e+08

Forest plot of the association of the *ACE I/D* polymorphism with essential hypertension. Pooled OR compared the *ID* genotype versus *II* genotype is shown under a random effects model. Studies are ordered by the publication year. CI, confidence interval; OR, odds ratio.

31 and 40 studies, respectively, for the two polymorphisms. The present meta-analysis differed from the previous ones [28,32] by focusing on a specific group with well defined genetic background, that is, Han Chinese population in this study. By doing this, we tried our best to exclude the confounding effect of population structure as well as ethnic differences. Furthermore, we included the most extensive association studies on these two polymorphisms by inclusion of recently published articles as well as all related articles published both in English and Chinese (see supplement material).

Staessen *et al.* [31] have conducted a meta-analysis in 1999 and found that the T235T genotype of the *AGT* gene was associated with 31% increase in the risk of essential hypertension as compared with the M235M

group in Caucasians instead of blacks and Asians. This result was partly confirmed by Mondry *et al.* [2] who have done a similar meta-analysis on Caucasian population. But for Asians, in Staessen *et al.*'s [31] article, only Japanese and Taiwanese populations were included, and the number of included participants were limited (*n* = 1995). In a meta-analysis [30] on Japanese population, the T allele of the *AGT* gene was associated with 60% increase in the risk of essential hypertension as compared with the M allele, which was quite different from the Staessen *et al.*'s [31] conclusion. Also, in another meta-analysis published in 2003, Chen *et al.* [28] concluded that T235T genotype might be associated with an increased risk of essential hypertension in Chinese population. However, only 853 patients and 835 controls from 10 studies were included in Chen *et al.*'s [28] analysis, so
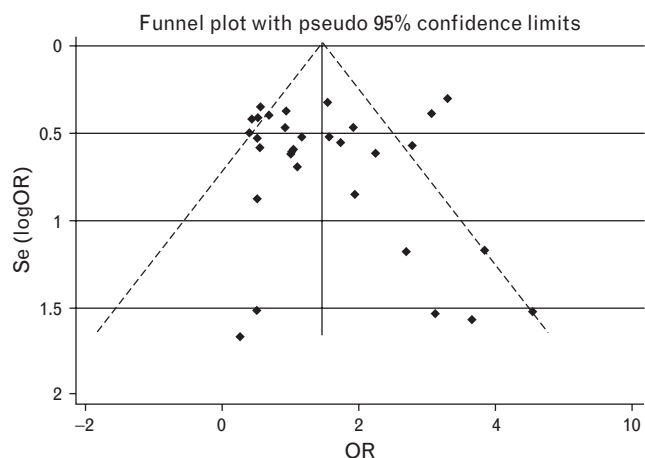
Fig. 7
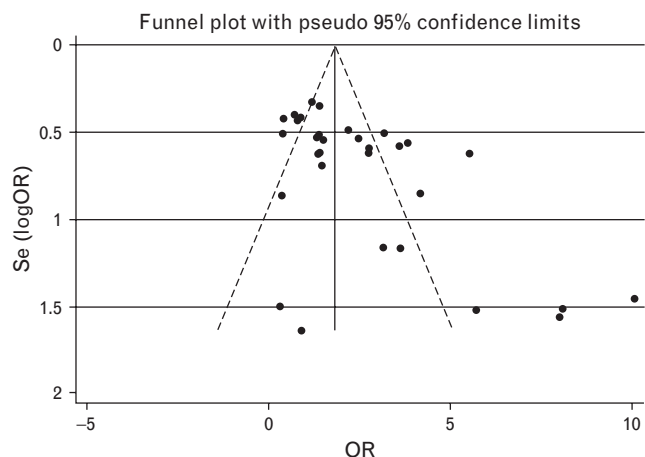
Funnel plots for studies investigating the effect of *AGT M235T* on the risk of essential hypertension. Horizontal axis represents the OR. Vertical axis represents the SE of log OR. Funnel plots are drawn with 95% confidence limits. OR, odds ratio; SE, standard error.

the conclusion should be taken with caution. On the other hand, from 2003, numerous studies have been carried out to examine this polymorphism in Chinese populations. In order to confirm whether *AGT M235T* is associated with essential hypertension in Han Chinese population, we did the current analysis including 31 studies from 13 provinces with 4727 patients and 3590 controls. The results strongly indicated that T allele and TT genotype of the *AGT* gene were significantly associated with increased risk of essential hypertension in Han Chinese population.

Fig. 8

Funnel plots for studies investigating the effect of *ACE I/D* on the risk of essential hypertension. Horizontal axis represents the OR. Vertical axis represents the SE of log OR. Funnel plots are drawn with 95% confidence limits. OR, odds ratio; SE, standard error.

The positive association of this polymorphism can be attributed to the polymorphism itself as well as other single-nucleotide polymorphisms (SNPs) within the same linkage disequilibrium bin. Haplotype analysis has revealed that *M235T* is in strong linkage disequilibrium with A-6G, A-20C, and G-217A, which are three important SNPs in the promoter region of *AGT* and have also been reported to be associated with hypertension [29]. Subsequently, we attempted to further figure out the genuine causal polymorphism by conducting additional meta-analysis of these three polymorphisms. Disappointedly, none of the three loci reached five articles by which number a meta-analysis can be done. Thus, we cautiously suggest that the association of *M235T* with essential hypertension in Han Chinese population may be attributed to the amino acid substitution itself. Also, it can serve as a genetic hallmark, whereas other SNPs within the same linkage disequilibrium bin such as A-6G, A-20C, and G-217A may influence the expression of *AGT* and regulation of the BP.

The association study results for *ACE I/D* polymorphism with essential hypertension were also differed in multiple ethnic groups. In one meta-analysis [16], including populations from Caucasians, blacks, and Asians, significant association was only observed in Asian population. Possible explanations can be attributed to ethnic differences as well as population structure, which can lead to a false positive/negative result [12]. The previous meta-analysis [32] on Chinese population indicated that *DD* genotype of *ACE* gene was associated with a 37% increase in the risk of having hypertension (95% CI 1.15–1.63, $P < 0.01$). However, the participants included in the analysis were not all from Han population. Moreover, literatures concerns with Chinese populations reported almost equal number of negative and positive results. Most of these studies investigated limited number of samples (less than 200 participants) in a tiny district. The conclusions were quite heterogeneous, with some studies reported *II* homozygote as well as *DD* homozygote in other studies was associated with essential hypertension [33]. Thus, it is very necessary to use meta-analysis to evaluate the genuine association between *I/D* polymorphism of *ACE* gene and essential hypertension in Han Chinese population. As mentioned in the 'Results' of the current meta-analysis, it included 40 studies from 18 provinces with 5820 patients and 5627 controls strongly indicated that *D* allele and *DD* genotype of the *ACE* gene were the risk factor of essential hypertension in Han Chinese population. The *I/D* polymorphism is located within intron 16 of the *ACE* gene, so it is very likely that it plays a regulatory role in the transcription and/or translation of this gene. Also, other polymorphisms in linkage disequilibrium with the *I/D* polymorphism may be the genuine causal one, by playing a role in the regulatory effect on ACE activity [14,34]. Therefore, after confirmation of this polymorphism with essential

hypertension in Han Chinese population, we suggest that *I/D* polymorphism as well as the SNPs within the same linkage disequilibrium bin can be finely explored for future study.

However, we should draw the conclusion cautiously for several limitations in this work. Absence of detailed data. We did not succeed in contacting all the authors of the studies included in the meta-analysis; therefore, we were unable to do further analysis. Although in most surveys the prevalence of hypertension appears to be equal in women and men [35], sex-specific effects of *ACE* gene on hypertension have been reported [2,36]. Among the collected studies, some reported that *DD* genotype might be associated with essential hypertension in women but not in total population. Thus, we planned to do meta-analysis with sex stratification. But most of the studies didn't stratify with sex, so we failed to do the stratified analysis. Too few samples in some studies. Almost half of the studies were composed of less than 100 patients or controls, and this defect may greatly affect the final result of this meta-analysis. Hospital-derived patients. The patients involved in this meta-analysis were all hospital-derived. We have collected several community-based large population-involved studies. But none of them followed the HWE, and were finally excluded from the analysis. Hospital-derived patients and poor design of the original studies may lead to heterogeneity of the pooled data.

Despite the limitations mentioned above, this meta-analysis strongly indicates that *AGT M235T* and *ACE I/D* polymorphisms contribute to the prevalence of essential hypertension in Han Chinese population. As these two polymorphisms might be only genetic markers, future works should be focused on these SNPs as well as polymorphisms within the same linkage disequilibrium bin. Furthermore, future association studies well designed for more specific information collection as well as with large sample size may greatly help us to further understand the genetic basis of hypertension.

Acknowledgements

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Supplement 1 Characteristics of published studies of the association between the *AGT* M235T polymorphism and essential hypertension in the meta-analysis

Author	Year	Region	Control			Case		
			TT	MT	MM	TT	MT	MM
Chiang *et al* [1]	1997	Taiwan	33	16	0	90	11	1
Cheung *et al* [2]	1998	Hongkong	73	24	3	77	21	1
Gong *et al* [3]	1998	Shandong	49	33	3	40	14	0
Liu *et al* [4]	1998	Beijing	52	53	8	69	26	8
Xiao *et al* [5]	1998	Jiangsu	36	24	2	58	17	0
Wang *et al* [6]	1999	Beijing	39	43	9	60	24	5
Guan et al [7]	2000	Shandong	35	37	10	37	39	7
Liu *et al* [8]	2000	Shanghai	32	53	12	48	37	5
Thomas *et al* [9]	2000	Hongkong	127	43	8	176	48	8
Li *et al* [10]	2001	Jiangsu	53	24	3	64	30	1
Wei *et al* [11]	2001	Shanghai	12	30	8	29	14	7
Sun *et al* [12]	2002	Chongqing	40	27	13	59	28	5
Wu *et al* [13]	2002	Shanghai	26	38	11	57	33	11
Yang *et al* [14]	2002	Henan	107	72	9	215	77	20
Kong *et al* [15]	2003	Henan	104	73	8	218	79	21
Lin *et al* [16]	2003	Sichuan	38	39	10	84	34	4
Liu *et al* [17]	2003	Anhui	36	35	8	32	21	5
Wang *et al* [18]	2003	Anhui	28	25	6	38	24	6
Zheng *et al* [19]	2003	Shanghai	42	18	2	39	22	5
Cai *et al* [20]	2004	Zhejiang	37	62	15	55	54	7
Kong *et al* [21]	2004	Henan	122	64	10	187	87	21
Wu *et al* [22]	2004	Taiwan	231	89	5	320	115	18
Zhang *et al* [23]	2005	Jiangsu	64	60	26	66	65	19
Dong *et al* [24]	2006	Guangdong	26	45	16	21	45	31
Liang *et al* [25]	2006	Guangdong	55	40	5	92	30	2
Zhang-Q *et al* [26]	2006	Henan	0	14	26	11	29	60
Zhang-S *et al* [27]	2006	Shanghai	50	35	3	58	22	0
Xing *et al* [28]	2007	Beijing	276	208	25	224	454	17
Yin *et al* [29]	2007	Sichuan	29	11	0	89	47	4
Wan *et al* [30]	2008	Beijing	7	21	14	31	112	25
Zhao *et al* [31]	2008	Yunnan	55	37	5	65	31	4

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Supplement 2 Characteristics of published studies of the association between the *ACE I/D* polymorphism and essential hypertension in the meta-analysis

Author	Year	Region	control			CASE		
			II	ID	DD	II	ID	DD
Jian et al [1]	1996	Beijing	61	50	22	50	54	18
Jeng et al [2]	1997	Taiwan	59	72	19	52	62	34
Zheng et al [3]	1997	Beijing	29	38	26	21	12	39
Hu et al [4]	1998	Beijing	28	46	21	20	61	40
Qiu et al [5]	1998	Beijing	61	78	26	17	48	69
Ye et al [6]	1998	Fujian	18	15	12	24	15	31
Zhou et al [7]	1998	Beijing	28	38	24	27	33	20
Li et al [8]	1999	Shanghai	57	86	35	61	72	26
Qiu et al [9]	1999	Zhejiang	42	43	17	25	29	14
Tan et al [10]	1999	Shanghai	21	29	13	16	14	12
Zhan et al [11]	1999	Jiangsu	28	63	21	32	46	34
Deng et al [12]	2000	Sichuan	40	50	23	42	27	35
Li et al [13]	2000	Shandong	53	89	41	29	68	57
Shan et al [14]	2000	Sichuan	42	53	12	48	66	16
Wang et al [15]	2000	Guangdong	23	21	8	34	29	13
Yuan et al [16]	2000	Liaoning	39	46	14	15	30	24
Zhou et al [17]	2000	Jilin	24	30	8	33	25	10
Gong et al [18]	2001	Shanghai	141	132	28	142	115	30
Li et al [19]	2001	Jiangsu	51	47	18	46	53	28
Wei et al [20]	2001	Shanghai	36	46	18	37	48	15
Gao et al [21]	2002	Shanxi	48	40	10	64	50	9
Shen et al [22]	2002	Fujian/Hubei/Xizang	18	27	15	6	44	38
Zhang et al [23]	2002	Liaoning	13	36	11	16	69	53
Zhang et al [24]	2002	Shandong	25	54	17	33	51	23
Kong et al [25]	2003	Henan	65	97	21	105	163	46
Wang et al [26]	2003	Guangdong	62	76	12	47	60	45
Dou et al [27]	2004	Beijing	22	110	102	31	111	92
Yan et al [28]	2004	Neimenggu	35	46	19	30	34	36
Liu et al [29]	2005	Shandong	21	50	29	13	43	44
Lv et al [30]	2005	Heilongjiang	42	46	19	44	42	16
Mu et al [31]	2005	Tianjin	51	44	21	19	49	37
Dong et al [32]	2006	Guangdong	23	47	17	34	44	19
Fang et al [33]	2007	Gansu	34	69	28	67	95	36
Gu et al [34]	2007	Jiangsu	16	17	5	19	19	18
He et al [35]	2007	Zhejiang	36	69	20	61	57	30
Zhang et al [36]	2007	Guangdong	33	45	18	24	51	40
Liang et al [37]	2008	Guangdong	56	50	16	30	18	16
Song et al [38]	2008	Shandong	54	41	14	28	43	20
Wu et al [39]	2008	Fujian	124	142	39	128	145	51
Zhang et al [40]	2008	Henan/Shandong	333	454	164	311	427	183

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