Methylenetetrahydrofolate reductase C677T gene polymorphism and essential hypertension: A meta-analysis of 10,415 subjects

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Abstract. The methylenetetrahydrofolate reductase (*MTHFR*) C677T gene polymorphism has been suggested to be associated with the risk of essential hypertension (EH), however, results remain inconclusive. To investigate this association, the present meta-analysis of 27 studies including 5,418 cases and 4,997 controls was performed. The pooled odds ratio (OR) and its corresponding 95% confidence interval were calculated using the random-effects model. A significant association between the *MTHFR* C677T gene polymorphism and EH was found under the allelic (OR, 1.32; 95% CI, 1.20-1.45; P=0.000), dominant (OR, 1.39; 95% CI, 1.25-1.55; P=0.000), recessive (OR, 1.38; 95% CI, 1.18-1.62; P=0.000), homozygote (OR, 1.59; 95% CI, 1.32-1.92; P=0.000), and heterozygote (OR, 1.32; 95% CI, 1.20-1.45; P=0.000) genetic models. A strong association was also revealed in subgroups, including Asian, Caucasian and Chinese. The Japanese subgroup did not show any significant association under all models. Meta-regression analyses suggested that the study design was a potential source of heterogeneity, whereas the subgroup analysis additionally indicated that the population origin may also be an explanation. Another subgroup analysis revealed that hospital-based studies have a stronger association than population-based studies, however, the former suffered a greater heterogeneity. Funnel plot and Egger's test manifested no evidence of publication bias. In conclusion, the present study supports the evidence for the association between the *MTHFR* C677T gene polymorphism and EH in the whole population, as well as in subgroups, such as Asian, Caucasian and Chinese. The carriers of the 677T allele are susceptible to EH.

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Introduction

Essential hypertension (EH), as a polygenic disease with a high prevalence rate, is considered to develop from a complex interaction of diverse environmental conditions with multiple genetic factors in which the underlying genetic mechanism remains unknown (1,2).

A number of studies have been conducted to investigate the genetic components of EH. The genes involved in classical systems, including Renin-angiotensin-aldosterone system (RAAS), the central nervous system and the vascular-endothelial system, have been previously focused on (3,4) and the homocysteine (Hcy) pathway is an emerging target. The methylenetetrahydrofolate reductase (*MTHFR*) C677T mutation is one of the most common gene polymorphisms. Mapped to chromosomal region 1p36.3, the *MTHFR* gene spans a 2.2-kb length with 11 exons and 10 introns. The product, known as *MTHFR*, is a critical enzyme in Hcy metabolism (5). The C677C to T mutation in the catalyzing region of the *MTHFR* gene may induce the displacement of alanine by valine. This change may lead to the thermolability of the enzyme and the inhibition of *MTHFR* activity, thus decreasing the transformation from 5,10-methyltetrahydrofolate to 5-methyltetrahydrofolate, which acts as a co-substrate for the conversion from Hcy to methionine (6). The level of plasma Hcy, as a result, increases and may lead to certain pathological changes, including vascular endothelial injury, vascular smooth muscle proliferation and nitric oxide production inhibition (7), which may lead to the development of hypertension, as well as other diseases (8-11).

Studies on the *MTHFR* C677T gene polymorphism and EH association have been extensively performed previously, but the results obtained remain controversial. In 2003, a study conducted in Spain suggested that the *MTHFR* TT genotypes can predict the development of EH in males, and the association may be mediated by the elevation of the Hcy level (12). In 2008 and 2012, Lin *et al* (13) and Yin *et al* (14) investigated the association among the Chinese population in the Taichung and Guangxi areas, respectively, and the studies indicated that the *MTHFR* 677C to T genotypes may be significant risk factors for EH. However, in 2012, a case-control study regarding the association between four Hcy pathway gene variants, including *MTHFR* 677T and EH in Caucasians, indicated no

significant association (15). More contrary results were found in a Japanese study by Nakata *et al* (16), which showed that the risk of hypertension significantly increased in subjects with the 677CC genotype, whereas the 677T gene polymorphism contributed to lower blood pressure.

Therefore, considering that the results of the studies carried out in the same ethnicity and different populations vary widely, the present meta-analysis of 10,415 subjects was conducted from 27 eligible studies to obtain a comprehensive conclusion on this association. The study demonstrated the association between the *MTHFR* C677T gene polymorphism and EH in the whole population. The association in different subgroup populations with reasonable clarification of the heterogeneity was shown clearly.

Materials and methods

Publication search and inclusion criteria. Electronic databases, including PubMed, Web of Science, China National Knowledge Infrastructure, WanFang and WeiPu were searched for relevant studies written in English or Chinese using the keywords: 'Methylenetetrahydrofolate reductase or *MTHFR*', 'polymorphism' and 'hypertension' (the last search was updated on April 1, 2013). Included studies were required to meet the following major criteria: i) Case-control or cross-sectional studies investigating the association between the *MTHFR* C677T polymorphism and EH; ii) present genotype counts of the patient and control groups; iii) diagnosis of EH patients based on the criteria of systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg. Secondary hypertension was excluded; and (iv) for overlapping studies, the studies with the larger sample size were included.

Data extraction. The following data from each eligible study were independently extracted in duplicate by two investigators: First author's name, publication year, population origin, genotype counts of cases and controls, study design, sample size, genotyping method, male percentage and age of patients and controls. The two extractions were compared and any discrepancy was resolved through discussion until a consensus was reached.

Statistical analysis. In the current meta-analysis, the association between the *MTHFR* C677T gene polymorphism and EH was revealed and analyzed by the odds ratio (OR) corresponding to the 95% confidence interval (CI) between cases and controls under five genetic models, which were the allelic (T/C), dominant (TT + CT/CC), recessive (TT/CT + CC), homozygote (TT/CC) and heterozygote models (CT/CC).

Between-study heterogeneity was estimated by χ^2-based Q analysis, and significance was set at P<0.05. The variation caused by heterogeneity was also evaluated by the inconsistency index, I^2 (I^2=0-25%, no heterogeneity; 25-50%, moderate; 50-75%, large; and 75-100%, extreme heterogeneity). The DerSimonian and Laird random-effects model was applied whether or not heterogeneity among studies was observed. The Z test was used to determine the pooled OR, and significance was set at P<0.05. In addition, to examine the degree to which an individual study affects the overall estimate, sensitivity analyses were conducted by removing one study at a time and
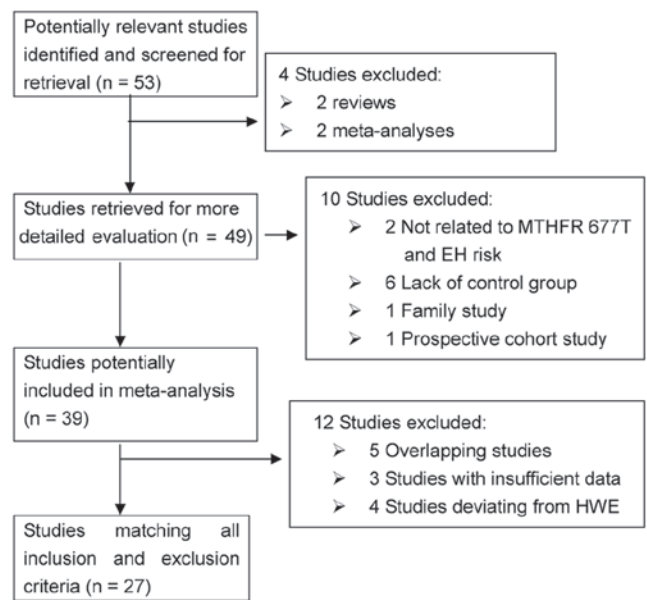

Figure 1. Flow diagram of the study selection process in the meta-analysis. Electronic databases, including PubMed, Web of Science, China National Knowledge Infrastructure (CNKI), WanFang and WeiPu, were searched for relevant studies that were written in English or Chinese, using the keywords 'methylenetetrahydrofolate reductase or MTHFR', 'polymorphism' and 'hypertension'. A total of 53 potentially relevant studies were obtained by the literature search (last study was updated on April 1, 2013), of which 27 met the inclusion criteria. Of the 26 excluded studies, four were reviews or meta-analyses, five were overlapping studies, six lacked a controls group, three did not provide genotype counts, two were unrelated to MTHFR 677T and essential hypertension (EH) risk and four studies deviated from the Hardy-Weinberg equilibrium (HWE). One family study and one prospective cohort study were also excluded.

analyzing the change of the pooled effect. To explore the potential sources of heterogeneity, a random-effects meta-regression was performed. Additionally, subgroup analyses were implemented subsequent to stratifying the included studies according to population origin and study design.

Fisher's exact test was used to assess the Hardy-Weinberg equilibrium (HWE). Potential publication bias was estimated by Begg's funnel plot together with Egger's linear regression test. Statistical analysis was performed using STATA 11.0 (StataCorp, College Station, TX, USA).

Results

Search results and study characteristics. A total of 53 potentially relevant papers were obtained by the literature search, of which 27 met the inclusion criteria (8,12-37). Of the 26 excluded studies, four studies were reviews or meta-analyses, five were overlapping studies, six lacked controls groups, three did not provide genotype counts, two were unrelated to *MTHFR* C677T and the EH risk and four studies deviated from the HWE. One family study and one prospective cohort study were also excluded (Fig. 1). The present meta-analysis involved 5,418 EH patients and 4,997 controls of Asian (Chinese, Japanese, Indian and Turks) and Caucasian (Australian, South Welsh, Argentinean, Austrian, Polish, Spanish and Czech) origin. Specifically, 6,188 subjects of 15 studies were Chinese from various parts of China, including Beijing, Tianjin, Shanghai, Fujian, Shenzhen,

Table I. Characteristics of 27 included studies of the association between the methylenetetrahydrofolate reductase C677T gene polymorphism and essential hypertension (EH).

First author (Refs.)	Year	Origin	Case			Control			EH/ control	Study design	Geno-typing	Male, %	EH age, years	Control age, years
			CC	CT	TT	CC	CT	TT						
Yin (14)	2012	Chinese	244	358	68	322	309	51	670/682	PB case-control	PCR-RFLP	0.494	M49.9±16.2 F48.3±15.8	M48.8±11.8 F47.1±11.2
Zhang (30)	2012	Chinese	128	53	8	117	41	7	189/165	PB case-control	PCR-RFLP	0.729	50.3±9.7	49.9±7.7
Cao (31)	2012	Chinese	65	105	53	49	68	30	223/147	HB case-control	PCR-RFLP	0.608	78.9±7.1	74.5±8.2
Liu (17)	2011	Chinese	58	70	27	74	47	19	155/140	HB case-control	PCR-RFLP	0.695	44.1±11.3	41.7±11.4
Liu (18)	2011	Chinese	54	59	33	61	39	12	146/112	HB case-control	PCR-RFLP	0.531	48.3±16.0	45.1±16.2
Cai (19)	2009	Chinese	77	44	9	31	7	1	130/39	HB case-control	PCR-RFLP	-	Total 61.8±9.8	
Luo (20)	2008	Chinese	260	151	31	138	51	6	442/195	HB case-control	PCR-RFLP	0.538	62.8±10.9	62.0±10.3
Lin (13)	2008	Chinese	19	27	4	73	44	6	50/123	HB case-control	PCR-RFLP	0.584	60.1±10.8	59.0±8.7
Xing (21)	2007	Chinese	202	309	184	182	222	105	695/509	HB case-control	PCR-RFLP	0.489	48.9±12.6	48.5±13.1
Tang (22)	2007	Chinese	139	93	20	138	51	6	252/195	HB case-control	PCR-RFLP	0.539	63.0±12.8	61.0±10.2
Li (32)	2006	Chinese	18	6	2	21	7	2	26/30	HB case-control	PCR-RFLP	0.536	60.3±11.3	55.5±13.5
Hu (33)	2006	Chinese	55	39	16	61	42	12	110/115	PB case-control	Sequenom	0.458	56.7±10.6	55.3±10.3
Liu (34)	2005	Chinese	29	45	26	31	50	19	100/100	PB case-control	PCR-RFLP	0.500	67.2±4.2	66.3±4.4
Wang (23)	2002	Chinese	17	51	37	14	23	9	105/46	HB case-control	PCR-RFLP	0.165	59.0~73.0	56.0~62.0
Zhan (28)	2000	Chinese	44	68	15	62	84	24	127/170	PB case-control	PCR-RFLP	0.468	57.9±9.9	50.6±10.5
Hui (35)	2007	Japanese	83	129	49	104	123	44	261/271	PB case-control	RTFQ-PCR	0.662	51.1±5.6	51.5±8.6
Lwin (29)	2006	Japanese	39	58	19	64	117	38	116/219	PB cross-sectional	PCR-RFLP	1.000	Total 53.1±8.9	
Nakata (16)	1998	Japanese	63	91	19	65	83	36	173/184	HB case-control	PCR-RFLP	-	-	-
Markan (25)	2007	Indian	105	40	8	105	28	0	153/133	HB case-control	PCR-RFLP	0.531	47.7±12.4	46.2±10.8
Ilhan (24)	2008	Turk	36	32	10	72	26	2	78/100	HB case-control	RTFQ-PCR	0.573	57.2±10.0	57.5±11.1
Fowdar (15)	2012	Australian	170	174	33	175	183	35	377/393	PB case-control	PCR-RFLP	0.452	63.1±10.9	61.0±10.5
Ng (26)	2009	South Welsh	14	14	10	40	32	8	38/80	PB case-control	PCR-RFLP	0.432	66.0~83.0	65.0~90.0
Fridman (27)	2008	Argentine	15	21	4	39	38	9	40/86	HB cross-sectional	PCR-RFLP	0.468	Total 45.2±16.8	
Tylicki (36)	2005	Austrian, Polish	40	39	11	42	38	10	90/90	HB case-control	PCR-RFLP	0.367	59.4±1.3	52.1±1.6
Heux (8)	2004	Australia	87	125	35	105	119	25	247/249	HB case-control	PCR-RFLP	0.562	Total 55.0±11.0	
Rodriguez (12)	2003	Spanish	83	115	34	95	100	20	232/215	PB case-control	PCR-RFLP	0.734	58.6±9.0	57.5±8.1
Benes (37)	2001	Czech	73	93	27	86	106	17	193/209	HB case-control	PCR-RFLP	0.761	55.6±11.5	54.9±11.6

Age is expressed as means ± standard deviation or the range. PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; RTFQ-PCR, real-time fluorescence quantitative PCR; Sequenom, Sequenom MassARRAY system; PB, population-based studies; HB, hospital-based studies; M, male; F, female; Total, total sample size; --, information unavailable.

Yunnan, Ningxia, Guangxi, Mongolia, Xinjiang and Taiwan (Table I).

Pooled effect under different genetic models. Among the 27 included studies, 14 separate studies showed a significant association between the *MTHFR* 677T gene polymorphism and EH risk (8,12-14,17-26), whereas the remaining 13 studies did not (15,16,27-37). Through meta-analysis, a significant association was revealed between the *MTHFR* 677T gene polymorphism and EH under the allelic (OR, 1.32; 95% CI, 1.20-1.45; P=0.000), dominant (OR, 1.39; 95% CI, 1.25-1.55; P=0.000), recessive (OR, 1.38; 95% CI, 1.18-1.62; P=0.000), homozygote (OR, 1.59; 95% CI, 1.32-1.92; P=0.000), and heterozygote (OR, 1.32; 95% CI, 1.20-1.45; P=0.000) genetic models (Figs. 2-5 and Table II). The overall pooled effect remained almost unchanged following the sensitivity analysis, which indicated the robustness of the results in the meta-analysis.

A subsequent subgroup analysis stratified by ethnicity was conducted in which a significant association between the *MTHFR* C677T gene polymorphism and EH was revealed in the Asian and Caucasian subgroups. The association was evident in all genetic models in the Asian subgroup (allelic: OR, 1.37; 95% CI, 1.21-1.54; P=0.000; dominant: OR, 1.47; 95% CI, 1.28-1.68; P=0.000; recessive: OR, 1.37; 95% CI, 1.12-1.68; P=0.002; homozygote: OR, 1.62; 95% CI, 1.27-2.05; P=0.000; and heterozygote: OR, 1.40; 95% CI, 1.25-1.56; P=0.000), whereas in the Caucasian subgroup an association was apparent under all models, except the heterozygote model (allelic: OR, 1.19; 95% CI, 1.05-1.36; P=0.007; dominant: OR, 1.19; 95% CI, 1.02-1.40; P=0.031; recessive OR, 1.42; 95% CI, 1.10-1.84; P=0.008; homozygote: OR, 1.53; 95% CI, 1.15-2.04; P=0.004; and heterozygote: OR, 1.13; 95% CI, 0.96-1.34; P=0.152). The association detected in the Asian group was stronger compared to the
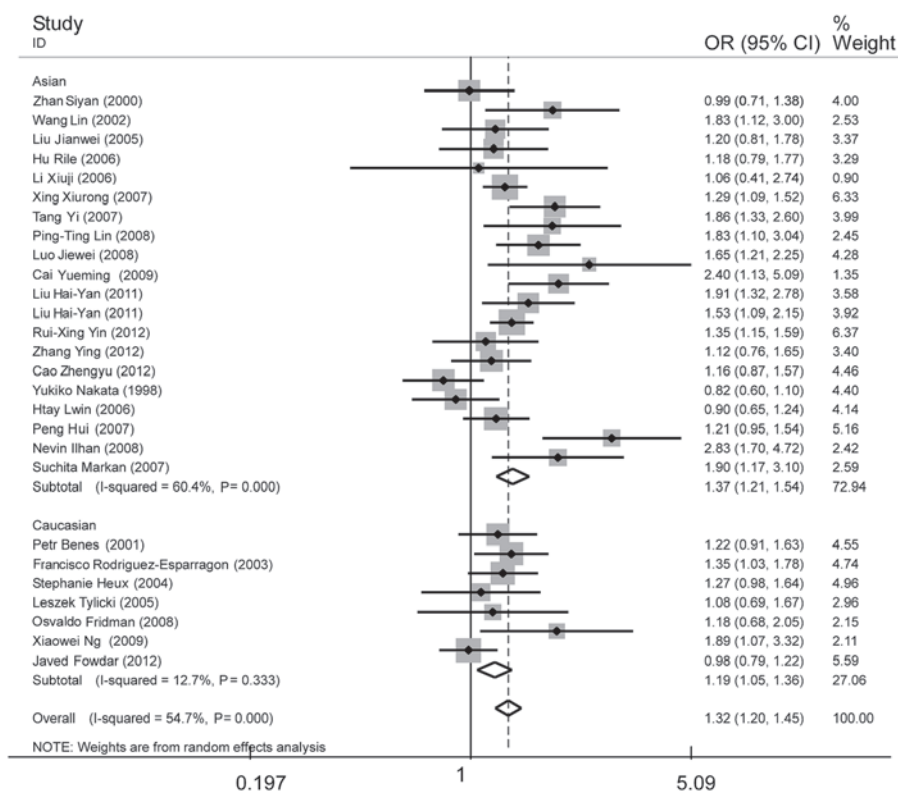
Figure 2. Forest plot of essential hypertension associated with the *MTHFR* C677T gene polymorphism under the allelic model (T/C). A significant association was revealed in the whole population (OR, 1.32; 95% CI, 1.20-1.45), Asian subgroup (OR=1.37; 95% CI, 1.21-1.54) and Caucasian subgroup (OR=1.19; 95% CI, 1.05-1.36). The heterogeneity test was significant in the whole population (I^2=54.7%, P=0.000) and Asian subgroup (I^2=60.4%, P=0.000), whereas the Caucasian subgroup showed no significant heterogeneity (I^2=12.7%, P=0.333). MTHFR, methylenetetrahydrofolate reductase; OR, odds ratio; CI, confidence interval.

Caucasian group under all genetic models, with the exception of the recessive model (Figs. 2-5 and Table II).

Heterogeneity analysis. The heterogeneity test was significant under the allelic (I^2=54.7%, P=0.000), dominant (I^2=35.6%, P=0.036) and homozygote (I^2=39%, P=0.021) models. Following the subgroup analysis, there was no significant heterogeneity observed in the Caucasian subgroup, whereas it remained apparent in the Asian subgroup (Figs. 2-5 and Table II). A further subgroup analysis in the Asian group was considered by stratification of Chinese, Japanese and others (Indian and Turk). Heterogeneity was not evident in any subgroup under the different genetic models, indicating that population origin may be one explanation for the between-study heterogeneity (Figs. 6-7 and Table III).

The further subgroup analysis also revealed a strong association between the *MTHFR* C677T gene polymorphism and EH in the Chinese population (allelic: OR, 1.39; 95% CI, 1.26-1.55; P=0.000; dominant: OR, 1.52; 95% CI, 1.34-1.71; P=0.000; recessive: OR, 1.45; 95% CI, 1.24-1.70; P=0.000; and homozygote: OR, 1.71; 95% CI, 1.44-2.03; P=0.000). None of these genetic models suggested any association between *MTHFR* 677T and EH among the Japanese population (Figs. 6-7 and Table III).

In addition, to explore other potential sources of heterogeneity, a random-effects meta-regression analysis including all 27 studies was conducted under the allelic, dominant and homozygote models. The publication year, population origin (Chinese as 1; Japanese as 2; other Asian, including Indian and Turk, as 3; and Caucasian as 4), case sample size, control sample size, total sample size, the ratio of case and control sample, the percentage of males, age of cases and controls, study design [hospital-based (HB) as 1 and population-based (PB) as 2] and genotyping method [polymerase chain reaction (PCR)-restriction fragment length polymorphism as 1; others, including Sequenom and real-time fluorescence quantitative-PCR, as 2] were considered as independent variables. In contrast to the subgroup analysis, no association of population origin was observed under any genetic model. However, a significant and marginal association of study design was revealed under the dominant (P=0.035) and allelic genetic models (P=0.068), respectively. Therefore, another stratified analysis was conducted by organizing studies into HB or PB studies under the dominant and allelic genetic models. There was no significant heterogeneity in the PB subgroup under the genetic models (Table IV), whereas the HB subgroup yielded a significance under the allelic genetic model indicating that study design may play a role in heterogeneity. Additionally, an association between the *MTHFR* C677T gene polymorphism and EH was found to be stronger in the HB studies compared to the PB studies.

Publication bias. Publication bias was assessed by Begg's funnel plot and Egger's test. The visible symmetrical funnel shape indicated no apparent evidence of publication bias in the meta-analysis, which is in accordance with the results of Egger's test (allelic: P=0.122; dominant: P=0.378, recessive: P=0.078, homozygote: P=0.104; and heterozygote genetic

Table II. Summary of the meta-analysis results of the association between the *MTHFR* C677T gene polymorphism and essential hypertension under the different genetic models.

Genetic model	Group	Pool OR (95% CI)	P-value	Heterogeneity I², %	Heterogeneity P-value	Studies number	Case size, n	Control size, n
Allelic	Whole population	1.32 (1.20-1.45)	0.000[a]	54.7	0.000[a]	27	5418	4997
	Asian	1.37 (1.21-1.54)	0.000[a]	60.4	0.000[a]	20	4201	3675
	Caucasian	1.19 (1.05-1.36)	0.007[a]	12.7	0.333	7	1217	1322
Dominant	Whole population	1.39 (1.25-1.55)	0.000[a]	35.6	0.036[a]	27	5418	4997
	Asian	1.47 (1.28-1.68)	0.000[a]	39.9	0.034[a]	20	4201	3675
	Caucasian	1.19 (1.02-1.40)	0.031[a]	0.0	0.654	7	1217	1322
Recessive	Whole population	1.38 (1.18-1.62)	0.000[a]	29.2	0.079	27	5418	4997
	Asian	1.37 (1.12-1.68)	0.002[a]	37.6	0.047[a]	20	4201	3675
	Caucasian	1.42 (1.10-1.84)	0.008[a]	2.6	0.406	7	1217	1322
Homozygote	Whole population	1.59 (1.32-1.92)	0.000[a]	39.0	0.021[a]	27	5418	4997
	Asian	1.62 (1.27-2.05)	0.000[a]	47.1	0.011[a]	20	4201	3675
	Caucasian	1.53 (1.15-2.04)	0.004[a]	10.2	0.351	7	1217	1322
Heterozygote	Whole population	1.32 (1.20-1.45)	0.000[a]	9.7	0.321	27	5418	4997
	Asian	1.40 (1.25-1.56)	0.000[a]	12.7	0.296	20	4201	3675
	Caucasian	1.13 (0.96-1.34)	0.152	0.0	0.878	7	1217	1322

[a]P<0.05. MTHFR, methylenetetrahydrofolate reductase; OR, odds ratio; CI, confidence interval.

Table III. Summary of the subgroup analysis results under different genetic models in different Asian populations.

Genetic model	Group	Pool OR (95% CI)	P-value	Heterogeneity I², %	Heterogeneity P-value	Studies number	Case size, n	Control size, n
Allelic	Chinese	1.39 (1.26-1.55)	0.000[a]	31.4	0.118	15	3420	2768
	Japanese	0.97 (0.76-1.25)	0.838	55.2	0.170	3	550	674
	Others (Indian and Turk)	2.30 (1.56-3.40)	0.000[a]	17.9	0.270	2	231	233
Dominant	Chinese	1.52 (1.34-1.71)	0.000[a]	13.7	0.300	15	3420	2768
	Japanese	1.05 (0.78-1.41)	0.755	32.6	0.227	3	550	674
	Others (Indian and Turk)	2.22 (1.28-3.83)	0.004[a]	43.6	0.183	2	231	233
Recessive	Chinese	1.45 (1.24-1.70)	0.000[a]	0.0	0.737	15	3420	2768
	Japanese	0.85 (0.51-1.40)	0.523	60.3	0.081	3	550	674
	Others (Indian and Turk)	8.58 (2.20-33.53)	0.002[a]	0.0	0.632	2	231	233
Homozygote	Chinese	1.71 (1.44-2.03)	0.000[a]	0.0	0.512	15	3420	2768
	Japanese	0.88 (0.50-1.56)	0.668	61.8	0.073	3	550	674
	Others (Indian and Turk)	11.30 (2.85-44.79)	0.001[a]	0.0	0.742	2	231	233

[a]P<0.05. OR, odds ratio; CI, confidence interval.

Table IV. Summary of the subgroup analysis results of all included studies stratified by study design.

Subgroup	Allelic genetic model OR (95% CI), P-value	Allelic genetic model Heterogeneity	Dominant genetic model OR (95% CI), P-value	Dominant genetic model Heterogeneity	Studies number	Case size, n	Control size, n
HB	1.45 (1.26-1.66), P=0.000[a]	I²=57.9%, P=0.002[a]	1.55 (1.34-1.79), P=0.000[a]	I²=31.6%, P= 0.104	17	3198	2587
PB	1.17 (1.05-1.31), P=0.006[a]	I²=31.6%, P=0.156	1.22 (1.06-1.42), P=0.007[a]	I²=24.0%, P=0.222	10	2220	2410
Overall	1.32 (1.20-1.45), P=0.000[a]	I²=54.7%, P=0.000[a]	1.39 (1.25-1.55), P= 0.000[a]	I²=35.6%, P=0.036[a]	27	5418	4997

[a]P<0.05. OR, odds ratio; CI, confidence interval; HB, hospital-based studies; PB, population-based studies.
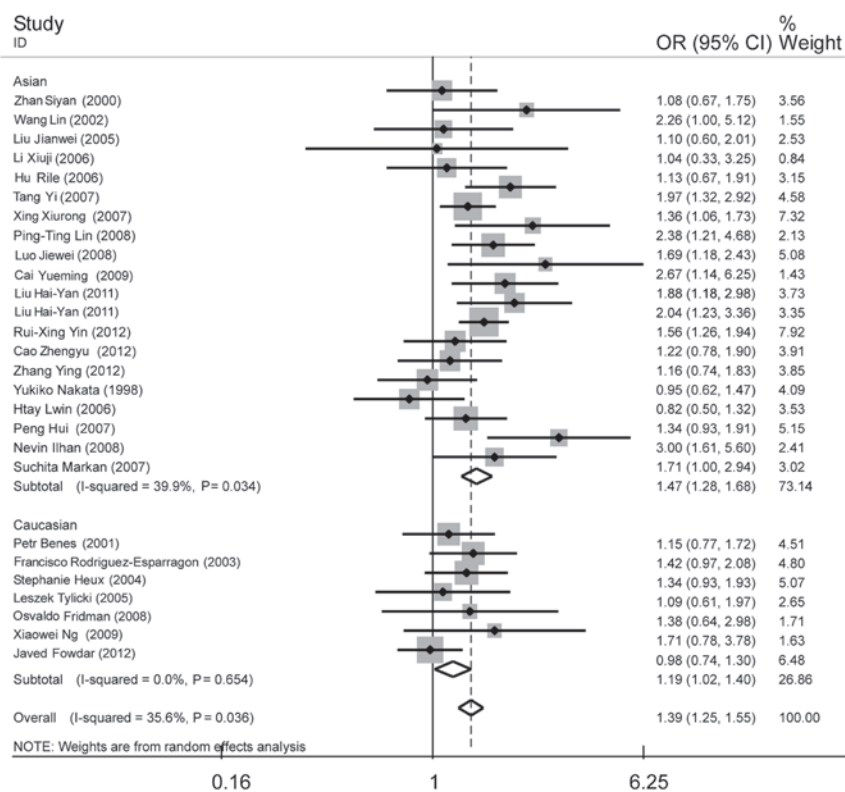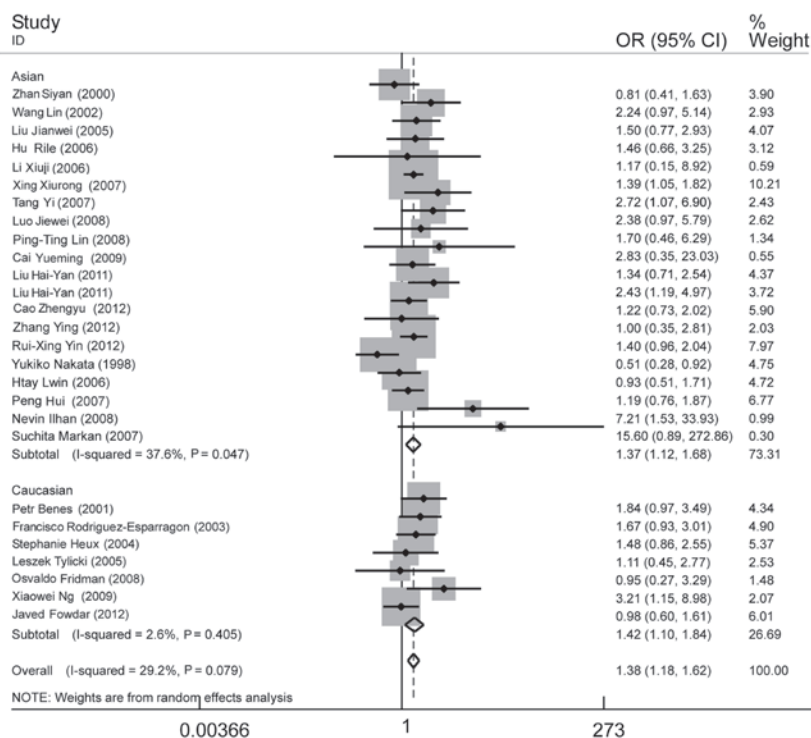
Figure 3. Forest plot of essential hypertension associated with the *MTHFR* C677T gene polymorphism under the dominant model (TT + CT/CC). A significant association was revealed in the whole population (OR, 1.39, 95% CI, 1.25-1.55), Asian subgroup (OR, 1.47, 95% CI, 1.28-1.68) and Caucasian subgroup (OR, 1.19, 95% CI, 1.02-1.40). A moderate heterogeneity was found in the whole population (I^2=35.6%, P=0.036) and Asian subgroup (I^2=39.9%, P=0.034), but did not existed in Caucasian subgroup (I^2=0.0%, P=0.654). MTHFR, methylenetetrahydrofolate reductase; OR, odds ratio; CI, confidence interval.

Figure 4. Forest plot of essential hypertension associated with the *MTHFR* C677T gene polymorphism under the recessive model (TT/CT + CC). A significant association was revealed in whole population (OR, 1.38; 95% CI, 1.18-1.62), Asian subgroup (OR, 1.37; 95% CI, 1.12-1.68) and Caucasian subgroup (OR, 1.42; 95% CI, 1.10-1.84). Only a marginal heterogeneity was apparent in the Asian subgroup (I^2=37.6%, P=0.047), whereas no significant heterogeneity was found in the whole population (I^2=29.2%, P=0.079) and Caucasian subgroup (I^2=2.6%, P=0.405). MTHFR, methylenetetrahydrofolate reductase; OR, odds ratio; CI, confidence interval.
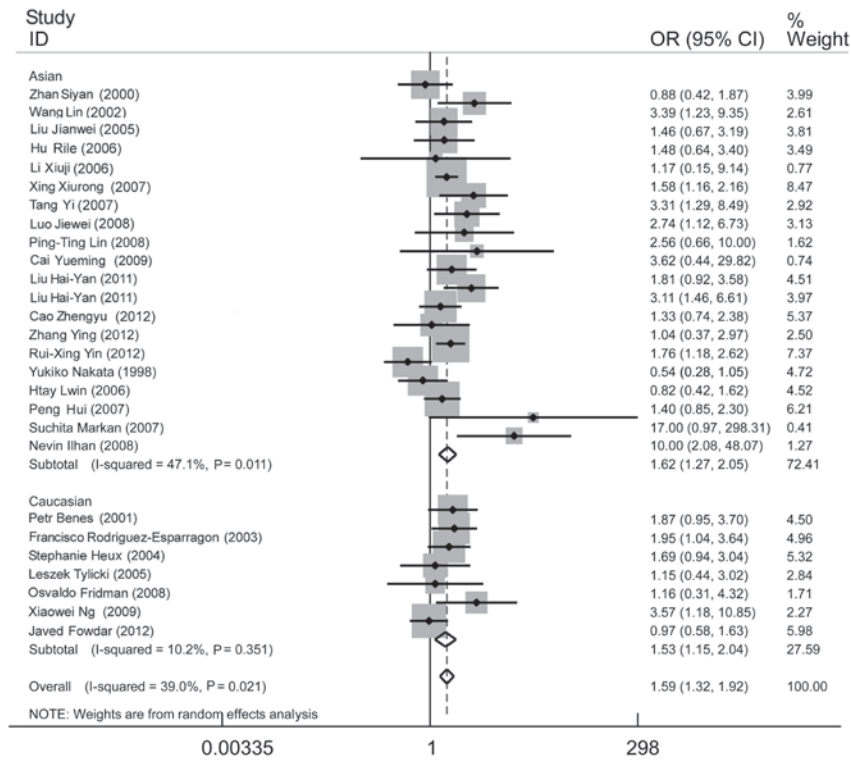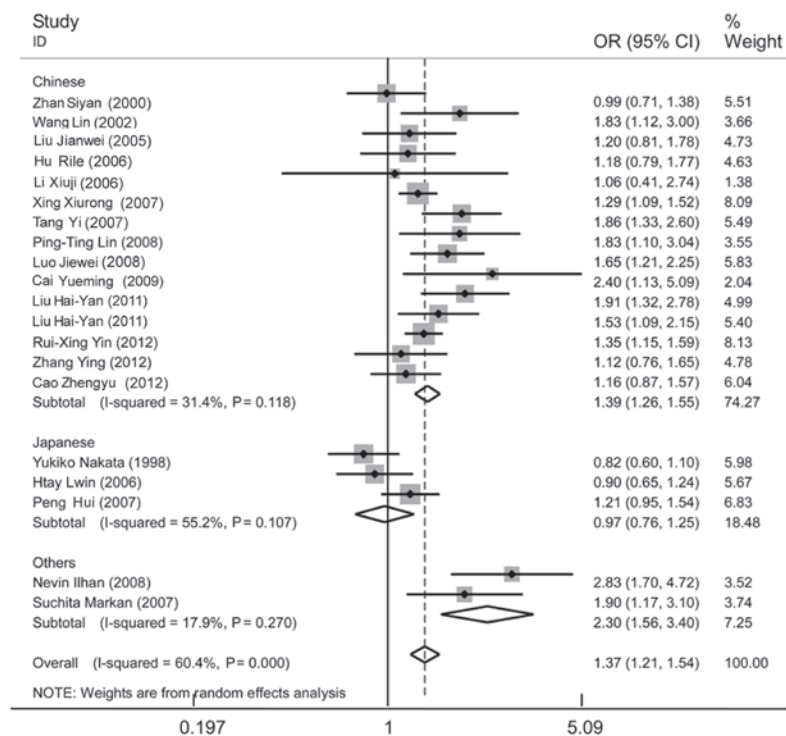
Figure 5. Forest plot of essential hypertension associated with the *MTHFR* C677T gene polymorphism under the homozygote model (TT/CC). A significant association was revealed in the whole population (OR, 1.59; 95% CI, 1.32-1.92), Asian subgroup (OR, 1.62; 95% CI, 1.27-2.05) and Caucasian subgroup (OR, 1.53; 95% CI, 1.15-2.04). The heterogeneity test was significant in the whole population (I^2=39.0%, P=0.021) and Asian subgroup (I^2=47.1%, P=0.011), whereas the Caucasian subgroup showed no significant heterogeneity (I^2=10.2%, P=0.351). MTHFR, methylenetetrahydrofolate reductase; OR, odds ratio; CI, confidence interval.

Figure 6. Forest plot of essential hypertension associated with the *MTHFR* C677T gene polymorphism in the Asian population under the allelic model (T/C) stratified by different populations, including Chinese, Japanese and others (Indian and Turk). A significant association was revealed in the Asian population (OR, 1.37; 95% CI, 1.21-1.54), as well as the Chinese (OR, 1.39; 95% CI, 1.26-1.55) and the others (OR, 2.3; 95% CI, 1.56-3.40) subgroups. No significant association was found in the Japanese subgroup (OR, 0.97; 95% CI, 0.76-1.25). A significant heterogeneity was detected in the Asian group (I^2=60.4%, P=0.000), but no longer existed in the Chinese (I^2=31.4%, P=0.118), Japanese (I^2=55.2%, P=0.107) and others (I^2=17.9%, P=0.270) subgroup, through subgroup analysis. MTHFR, methylenetetrahydrofolate reductase; OR, odds ratio; CI, confidence interval.
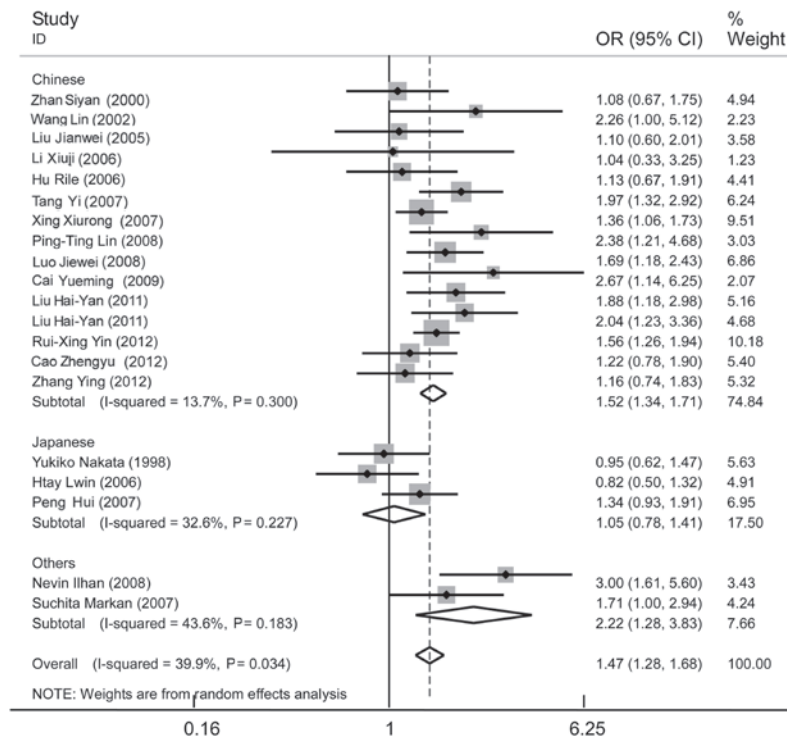
Figure 7. Forest plot of essential hypertension associated with the *MTHFR* C677T gene polymorphism in the Asian population under the dominant model (TT + CT/CC) stratified by different populations; Chinese, Japanese and others (Indian and Turk). A strong association was revealed in the Asian population (OR, 1.47; 95% CI, 1.28-1.68), as well as the Chinese (OR, 1.52; 95% CI, 1.34-1.71) and others (OR, 2.22; 95% CI, 1.28-3.83) subgroups. No significant association was found in the Japanese subgroup (OR, 1.05; 95% CI, 0.78-1.41). Moderate heterogeneity was apparent in the Asian population (I^2=39.9%, P=0.034), whereas no significant heterogeneity was detected in the Chinese (I^2=13.7%, P=0.300), Japanese (I^2=32.6%, P=0.227) and others (I^2=43.6%, P=0.183) subgroups. MTHFR, methylenetetrahydrofolate reductase; OR, odds ratio; CI, confidence interval.
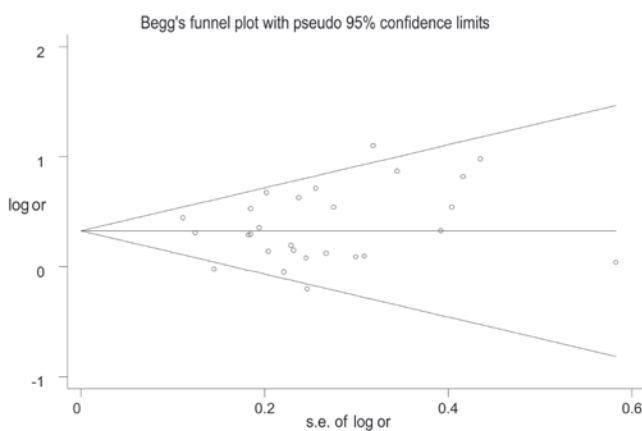

Figure 8. Begg's funnel plot for studies of the association between the *MTHFR* C677T polymorphism and EH under the dominant genetic model (CT + TT/CC). Begg's funnel plot was used to assess the publication bias. The vertical axis is log OR and the horizontal axis is the standard error (s.e.) of the log OR. The visible symmetrical funnel shape shows no apparent evidence of the publication bias in the meta-analysis. MTHFR, methylenetetrahydrofolate reductase; EH, essential hypertension; OR, odds ratio.

model: P=0.550). There was no significant difference revealed, which suggests a low probability of publication bias (Fig. 8).

Discussion

Meta-analysis is an efficient method to obtain a pooled effect of various studies with relatively small sample sizes

and to achieve more comprehensive conclusions. Several meta-analyses that have been conducted in our Department of Geriatrics (First Affiliated Hospital of Nanjing Medical University, Nanjing, China) recently provided reliable results for the association between different gene polymorphisms and cardiovascular diseases (38,39). The present meta-analysis, which involved 5,418 cases and 4,997 controls from 27 studies carried out worldwide, indicated a strong association between the *MTHFR* C677T gene polymorphism and EH in the whole population under the allelic (OR, 1.32; 95% CI, 1.20-1.45; P=0.000), dominant (OR, 1.39; 95% CI, 1.25-1.55; P=0.000), recessive (OR, 1.38; 95% CI, 1.18-1.62; P=0.000), homozygote (OR, 1.59; 95% CI, 1.32-1.92, P=0.000), and heterozygote (OR, 1.32; 95% CI, 1.20-1.45; P=0.000) genetic models.

A significant association was detected in the Asian and Caucasian groups (P<0.05) suggesting that carriers of 677T or 677TT in these populations may suffer a significantly increased risk of developing EH. In the further subgroup analysis of the Asian population, stratified as Chinese, Japanese and others (Indian, Turk), the significant heterogeneity no longer existed in any subgroup suggesting that the population origin may contribute to heterogeneity. Future studies should pay increasing attention to the possible differences in genetic background of various populations. In addition, the pooled analysis of 6,188 Chinese subjects from 15 studies showed a strong association between the *MTHFR* C677T gene polymorphism and EH, revealing that it was a possible risk factor for EH in the Chinese population. There was no apparent association between the *MTHFR* C677T gene polymorphism

and EH among the Japanese population. Considering that only three studies were included in the subgroup, a meta-analysis including more Japanese studies and future studies with larger samples could be conducted to confirm this result. Additionally, the study design was identified as an explanation for between-study heterogeneity in the meta-regression analysis, as well as the subgroup analysis. A stronger association together with greater heterogeneity was observed in HB studies compared to PB studies. This difference may be explained by the fact that control subjects from hospitals tend to have more complicated health states and confounding factors, whereas the controls selected from the general population would be more representative. The poor comparability between patients and controls in HB studies may contribute to the heterogeneity and exaggerate the association of the *MTHFR* 677T gene polymorphism and EH to a certain degree (40).

Two meta-analyses, by Qian *et al* (41) and Niu *et al* (42), were conducted previously in the whole population and in the Chinese population, respectively. Compared to these studies, the present meta-analysis had substantial advantages in the following aspects: First, only nine studies with 1,520 patients and 1,334 controls in the study by Niu *et al* (42) and 12 out of 25 studies in the study by Qian *et al* (41) were studies regarding the association between the *MTHFR* C677T gene polymorphism and EH. The remaining studies in the two meta-analyses assessed hypertension in pregnancy (HIP). Considering the multiple pathogenesis and complicated physiopathological changes in HIP cases, it is not suitable to analyze the pooled effect of the association between the *MTHFR* C677T gene polymorphism and hypertension with HIP patients. Therefore, with an increase of 18 studies from the latest meta-analysis regarding the association between *MTHFR* C677T and EH (42), the present study assessed 27 eligible studies with a total of 5,418 EH cases and 4,997 controls. This provides more reliable results with less clinical heterogeneity. One overlapping study and particular studies of secondary hypertension, which were included previously, were excluded. Second, compared to the unexplained heterogeneity of the Asian subgroup in the Qian *et al* (41) study, the heterogeneity of the Asian population no longer existed in the Chinese, Japanese and other (Indian and Turk) subgroups following stratified analysis in the present study. This suggested that the population origin may be one reason for heterogeneity. Third, in terms of meta-regression, while only a marginal association (P=0.082) of study design was observed in the Niu *et al* (42) study, the association in the present study was significant (P=0.035). This may be due to the relatively few included studies in the study by Niu *et al*, and studies involved in meta-regression should usually be ≥10 (43). Therefore, with stronger evidence, the present meta-analysis demonstrated the effect of study design in heterogeneity. Finally, the present meta-analysis involved a wide range of various populations and the pooled effect of 15 Chinese studies with 3,420 cases and 2,768 controls was also revealed in the subgroup analysis. For this reason, the study renewed the two previous meta-analyses with additional supplements and appropriate adjustments, and thus, to the best of our knowledge, becoming the most comprehensive meta-analysis regarding the association between *MTHFR* C677T and EH.

The present meta-analysis, although notably having advantages, such as large sample sizes, reliable statistical results and sound heterogeneity management, has certain limitations. First, large-scale studies on the association between the *MTHFR* gene polymorphism and EH are inadequate. Second, as discussed previously, the *MTHFR* C677T gene mutation is postulated to play a role in developing EH through generating hyperhomocysteinemia. However, the level of plasma Hcy is also influenced by certain environmental factors, including lack of folate and vitamin B6 in the diet, and is associated with plasma folate levels (8,26). The interaction between gene and environment was not taken into account. Therefore, future prospective cohort studies with large sample sizes should be performed to confirm these results and studies should include these considerations.

In conclusion, the present meta-analysis, including 27 studies with 10,415 subjects, indicates that the *MTHFR* C677T gene polymorphism is associated with increased EH risks in different populations as a whole, as well as in separate subgroups, such as Asian, Caucasian and Chinese. Carriers of the 677T allele are susceptible to EH. *MTHFR* C677T should therefore be tested for as a predictive screening for the identification of the individuals that may develop EH in the future. As an improvement on the previous studies with noteworthy reinforcement and development, the present results may potentially provide a stronger foundation for the identification of the mechanism through which the *MTHFR* gene may play a role in developing EH. More studies should be conducted to confirm the conclusion, and polymorphisms within the same linkage disequilibrium area should be focused on as well.

Acknowledgements

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