Hypertension Research (2012) 35, 1129–1134

www.nature.com/hr

REVIEW

Meta-analysis of the methylenetetrahydrofolate reductase C677T polymorphism and susceptibility to pre-eclampsia

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A number of studies have investigated the association between the methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and the risk of pre-eclampsia (PE) in various populations and have delivered inconsistent results. Therefore, this meta-analysis of 36 case–control studies, comprising 4253 PE cases and 4950 controls, were assessed to evaluate a possible association. The pooled results showed that the *MTHFR C677T* polymorphism was significantly associated with PE ($P = 0.03$, odds ratio (OR) = 1.25, 95% confidence interval (CI) = 1.02–1.54, for the additive comparison; $P = 0.04$, OR = 1.14, 95% CI = 1.01–1.29, for the dominant genetic model). The results of the subgroup analysis showed that MTHFR 677T had the effect of increasing the PE risk for the recessive genetic model ($P < 0.0001$, OR = 1.76, 95% CI = 1.33–2.33, $P_{heterogeneity} = 0.28$), the additive comparison ($P = 0.002$, OR = 2.09, 95% CI = 1.31–3.31, $P_{heterogeneity} = 0.08$) and allele contrasts ($P = 0.03$, OR = 1.42, 95% CI = 1.04–1.95, $P_{heterogeneity} = 0.0001$) in the Asians, while no evidence of an association between *MTHFR C677T* polymorphisms and PE was observed in the Caucasians. This meta-analysis suggests that the *MTHFR C677T* polymorphism is capable of causing PE susceptibility in the Asians but not in the Caucasians.
Hypertension Research (2012) **35,** 1129–1134; doi:10.1038/hr.2012.117; published online 23 August 2012

Keywords: methylenetetrahydrofolate reductase; MTHFR C677T; polymorphism

INTRODUCTION

Pre-eclampsia (PE) is a major pregnancy complication associated with increased blood pressure and proteinuria after 20 weeks of gestation.[1] Although this disorder is a significant cause of maternal and perinatal morbidity and mortality, its etiology is not yet clear. A genetic susceptibility to PE has been well established, and genes involved with endothelial dysfunction, oxidative stress, angiogenesis and thrombophilia have been associated with PE.[2–4]

Methylenetetrahydrofolate reductase (MTHFR) is an enzyme that catalyzes the conversion of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a co-substrate for the transmethylation of homocysteine to methionine. Severe MTHFR deficiency is associated with hyperhomocysteinemia. The human *MTHFR* gene located on chromosome 1p36.3 is 1980-bp long and has a common polymorphism, C677T. This C to T transition in exon 4 results in the substitution of alanine with valine (A222V) in the N-terminal catalytic domain, which renders the enzyme thermolabile and elevates homocysteine levels.[5] In clinical studies, hyperhomocysteinemia has been associated with pregnancy complications such as recurrent pregnancy loss, pregnancy-induced hypertension, placental abruption and PE. Therefore, the *MTHFR C677T* gene polymorphism may present a genetic risk factor for PE.[6–9]

To date, numerous case–control studies have been conducted to investigate the role of the *C677T* gene polymorphism in the development of PE, but these studies have produced conflicting or inconclusive results. The published studies have generally been restricted in sample size and ethnic diversity, and individual studies may have insufficient power to achieve a comprehensive and reliable conclusion. In 2004, Kosmasa *et al.*[10] performed a meta-analysis to clarify the effect of the *MTHFR C677T* gene on the risk of PE. The meta-analysis only contained 22 studies and did not perform the subgroup analysis by ethnicity. Since 2004, there has been an expanding body of published literature assessing the association between the *MTHFR C677T* polymorphism and PE. To perform a more comprehensive estimate of the association between the *MTHFR C677T* polymorphism and the risk of PE, we conducted a meta-analysis to assess the association between the *MTHFR C677T* polymorphism and PE susceptibility.

METHODS

Search strategy

A comprehensive search was performed to search the electronic databases of PubMed, Web of Science, the Chinese Biomedical Database and the Chinese National Knowledge Infrastructure with the following terms: MTHFR,

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Received 14 April 2012; revised 31 May 2012; accepted 1 June 2012; published online 23 August 2012

polymorphism, PE and PE. The publication languages included only English and Chinese. The retrieved abstracts were read to identify studies examining the genotype association between the *MTHFR C677T* polymorphism and PE. In addition, all references cited were reviewed to identify additional studies. An upper date limit of March 2012 was applied; however, no lower date limit was set. When more than one of the same patient population was included in more than one publication, only the most recent or complete study was included in the meta-analysis.

Inclusion criteria

We reviewed the titles and abstracts of all citations and retrieved literature. The following inclusion criteria were used for literature selection:[1] the publication was a case–control study referring to the association between the *MTHFR C677T* polymorphism and PE;[2] the cases consisted of PE patients and controls consisted of normotensive pregnant women;[3] there was no deviation from the Hardy–Weinberg equilibrium (HWE) among the controls; and[4] the papers offered sample sizes, distributions of alleles, genotypes or other information to help us infer results. When the genotype frequency was not reported, we contacted the author to obtain the relevant information by e-mail. Major reasons for exclusion of the studies were[1] lack of controls,[2] duplication[3] and insufficient reported data.

Data extraction

Data were extracted from each study by two independent reviewers (XP Xia and WW Chang) according to the selection criteria. Decisions were compared, and disagreements about the study selection were resolved by consensus or by involving a third reviewer (YX Cao). The following information was extracted from the literature: first author, year of publication, ethnicity of the study population, number of cases and controls and distribution of genotypes and alleles in all groups. The ethnicities were categorized as Asians, Caucasians and others.

Statistical analysis

Crude ORs with their 95% CIs were used to assess the strength of association between the *MTHFR C677T* polymorphism and PE risk. The pooled ORs were calculated for the allele contrasts (T *vs.* C), dominant genetic model (TT + TC *vs.* CC), recessive genetic model (TT *vs.* TC + CC) and additive comparison (TT *vs.* CC). The subgroup analysis was also performed by ethnicity. In our study, two meta-analysis models were applied for dichotomous outcomes according to the results of heterogeneity tests among the individual studies: the fixed effects model (Mantel and Haenszel)[11] and the random-effects model (DerSimonian and Kacker).[12] The heterogeneity assumption was assessed by a χ^2-based *Q*-test and an I^2 test.[13] The heterogeneity was considered statistically significant if $P<0.10$. The random-effects model (if $P<0.10$) or the fixed effects model (if $P\geqslant 0.10$) was used to summarize the combined odds ratio (OR). The significance of the pooled OR was determined by the *Z*-test. A *P*-value of <0.05 was considered significant. Deviation from the HWE ($P<0.05$) among the control groups within each study was assessed by an exact test using an online HWE calculator (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). A possible publication bias was tested by Begg's funnel plot and Egger's test.

Analyses were performed using the Stata software version 10 (StataCorp LP, College Station, TX, USA), and Review Manager 4.2 (Cochrane Collaboration, http://www.cc-ims.net/RevMan/relnotes.htm). All *P*-values presented are two-tailed.

RESULTS

Study characteristics

Through a literature search, we identified 40 potentially eligible studies. Among the 40 eligible articles, four studies were excluded because the genotype distribution in the control population deviated significantly from the HWE.[14–17] There were four overlapping case–control studies in two publications,[18–21] and we chose the most recent studies.[19,20] Thus, 34 reports were eligible.[19,20,22–53] One report included separate data on three hypertensive populations.[35] Overall, 36 study comparisons were included in the meta-analysis (Table 1). Out of all eligible studies, 9 studies were conducted on Asians, 18 studies on Caucasians and 9 studies on others. Our final pool of eligible studies included 36 studies with 4253 cases and 4950 controls (Table 2). The characteristics of the studies included in the meta-analysis are listed in Table 1.

Meta-analysis results

Global statistical results. Table 2 lists the main results of this meta-analysis. The random-effects model was used to pool the results, as there was significant heterogeneity in 36 studies in the genetic model (Table 2). Using the random-effects model, the 677T polymorphism

Table 1 Characteristics of case–control studies included in *MTHFR C677T* polymorphism and PE

		Cases			Controls		
First author (year)	*Country (ethnicity)*	*TT*	*TC*	*CC*	*TT*	*TC*	*CC*
Sohda (1997)	Japan (Asian)	19	32	16	38	49	11
Chikosi (1999)	South Africa (African)	86	18	1	97	13	0
Grandone (1999)	Italy (Caucasian)	28	25	41	41	64	24
O'Shaughnessy (1999)	UK (Caucasian)	138	114	31	51	37	12
Powers (1999)	USA (Caucasian)	35	49	15	54	46	14
Kobashi (2000)	Japan (Asian)	25	40	8	83	99	33
Laivuori (2000)	Finland (Caucasian)	64	45	4	56	40	7
Li (2000)	China (Asian)	9	30	18	44	58	18
Rajkovic (2000)	Zimbabwe (African)	142	28	1	151	32	0
Kaiser (2001)	Australia (Caucasian)	71	66	19	37	31	11
Kim (2001)	USA (Caucasian)	131	117	33	167	152	41
Lachmeijer (2001)	Netherlands (Caucasian)	22	21	4	58	51	11
Raijmakers (2001)	Netherlands (Caucasian)	72	74	21	205	162	36
Watanabe (2001)	Japan (Asian)	40	59	34	89	103	32
Morrison (2002)	UK (Caucasian)	169	193	42	81	66	17
Prasmusinto (2002)	Indonesian (Asian)	34	6	1	22	5	0
Prasmusinto (2002a)	Croatian (Caucasian)	11	12	2	18	15	5
Prasmusinto (2002b)	German (Caucasian)	7	7	1	12	15	7
De Maat (2004)	Netherlands (Caucasian)	78	59	20	63	75	19
Perez-Mutul (2004)	Mexica (Mexican)	33	66	49	36	80	61
Pegoraro (2004)	South Africa (African)	232	38	1	298	38	2
Williams (2004)	Peru (South American)	37	61	25	62	85	30
Yilmaz (2004)	Turkey (Caucasian)	29	28	7	24	17	6
Also-Rallo (2005)	Spain (Caucasian)	11	24	8	37	56	29
Davalos (2005)	Mexica (Mexican)	13	14	6	24	27	11
Dalmaz (2006)	Brazil (Mixed)	31	27	17	76	51	18
Jaaskelainen (2006)	Finland (Caucasian)	78	43	12	64	42	6
Dusse (2007)	Brazil (Mixed)	16	12	2	45	31	7
Zhang (2007)	China (Asian)	12	21	20	10	30	9
Nagy (2007)	Hungary (Caucasian)	49	43	9	32	35	6
Canto (2008)	Mexica (Mixed)	36	66	23	61	131	82
Muetze (2008)	German (Caucasian)	30	34	7	35	29	15
Zhang (2008)	China (Asian)	22	21	7	29	8	3
Shen (2009)	China (Asian)	12	35	14	30	21	9
Stiefel (2009)	Spain (Caucasian)	27	157[a]		21	113[b]	
Aggarwal (2011)	India (Asian)	160	33	7	134	58	8

Abbreviation: PE, pre-eclampsia.
[a]TC + CC in the PE group.
[b]TC + CC in the control group.

Table 2 Summary ORs and 95% CI of *MTHFR C677T* polymorphism and PE

Analysis model	Populations (study no.)	OR (95% CI)	P_{OR}	I^2 (%)	P_H
T *vs.* C	Total[35]	1.11 (0.99–1.25)[a]	0.08	59.8	<0.0001
	Caucasian[17]	1.02 (0.89–1.18)[a]	0.73	48.6	0.01
	Asian[9]	1.42 (1.04–1.95)[a]	0.03	74.2	0.0001
	Others[9]	1.06 (0.88–1.29)[a]	0.54	43.8	0.08
TT *vs.* (TC + CC)	Total[35]	1.19 (0.99–1.44)[a]	0.07	38.9	0.01
	Caucasian[17]	1.07 (0.84–1.37)[a]	0.58	32.9	0.09
	Asian[9]	1.76 (1.33–2.33)	<0.0001	18.8	0.28
	Others[9]	0.95 (0.74–1.23)	0.69	29.5	0.18
(TT + TC) *vs.* CC	Total[36]	1.14 (1.01–1.29)[a]	0.04	38.3	0.01
	Caucasian[18]	1.08 (0.96–1.22)	0.21	0	0.78
	Asian[9]	1.52 (0.94–2.46)[a]	0.09	77.0	<0.0001
	Others[9]	1.09 (0.90–1.31)	0.40	0	0.51
TT *vs.* CC	Total[35]	1.25 (1.02–1.54)[a]	0.03	36.8	0.02
	Caucasian[17]	1.12 (0.92–1.37	0.27	0	0.52
	Asian[9]	2.09 (1.31–3.31)[a]	0.002	42.4	0.08
	Others[9]	0.99 (0.73–1.33)	0.93	35.2	0.14

Abbreviations: CI, confidence interval; OR, odds ratio; PE, pre-eclampsia.
I^2 (%) and P_H value for heterogeneity.
[a]Estimates for random effects model P_{OR}. The *P*-value of OR determined by the *Z*-test.

had an effect of increasing the PE risk under the additive comparison ($P = 0.03$, OR = 1.25, 95% confidence interval (CI) = 1.02–1.54, $P_{\text{heterogeneity}} = 0.02$) and the dominant genetic model ($P = 0.04$, OR = 1.14, 95% CI = 1.01–1.29, $P_{\text{heterogeneity}} = 0.01$). There was no significant association between the 677T polymorphism and PE risk under the recessive genetic model and the allele contrasts (Table 2).

Stratification analysis. We only performed the subgroup analysis of ethnicity on the Asian and Caucasian populations. The results showed that the *MTHFR C677T* polymorphism increased the PE risk for the recessive genetic model ($P < 0.0001$, OR = 1.76, 95% CI = 1.33–2.33, $P_{\text{heterogeneity}} = 0.28$), the additive comparison ($P = 0.002$, OR = 2.09, 95% CI = 1.31–3.31, $P_{\text{heterogeneity}} = 0.08$) and allele contrasts ($P = 0.03$, OR = 1.42, 95% CI = 1.04–1.95, $P_{\text{heterogeneity}} = 0.0001$) in the Asians, whereas no evidence of an association between *MTHFR C677T* polymorphisms and PE was observed in the Caucasians (Table 2 and Figure 1). The sensitivity analysis showed that the Aggarwal *et al.* study was the origin of the heterogeneity. When this article was excluded, the heterogeneity in all models disappeared, and an obvious association was noted for Asians under the dominant genetic model ($P = 0.002$, OR = 1.79, 95% CI = 1.25–2.58, $P_{\text{heterogeneity}} = 0.35$).

Publication bias. As shown in Figure 2, the shape of the funnel plot did not reveal obvious asymmetry under the recessive genetic model. Egger's test was then used to provide statistical evidence of funnel plot symmetry. The results still did not suggest any evidence of a publication bias (data not shown).

DISCUSSION

During pregnancy, there are several hypertensive disorders that complicate gestation, ranging from mild and transient hypertension to severe PE/eclampsia syndrome.[54] PE is a hypertensive disorder that is characterized by high blood pressure and proteinuria, and it occurs in the second or third trimester of pregnancy with a particularly high incidence in the Caucasian population.[1,7,55] It is known that a woman who has a pregnancy complicated by PE has an increased risk of hypertension, ischemic heart disease and stroke in the 10–15 years after the pregnancy.[56] As PE affects 2–5% of pregnancies, 1.2–3 million people in the United kingdom and 6–15 million in the United States of America are offspring of pregnancies complicated by PE.[57,58]

PE is a complex, multifactorial pregnancy-specific condition involving genetic, environmental and behavioral factors.[59] Hyperhomocysteinemia has been associated with the development of PE, and the most common polymorphism associated with this condition is the thermolabile 677T allele of the *MTHFR* gene, which results in reduced enzyme activity and impaired homocysteine/folate metabolism, leading to moderate hyperhomocysteinemia.[5,60,61] The literature on the relationship between the *MTHFR C677T* polymorphism and PE risk is replete with small studies reporting controversial findings, and no clear consensus has been reached. We therefore performed a meta-analysis to form a more precise estimation of the association of the *MTHFR C677T* polymorphism and PE risk.

Our meta-analysis was based on 36 studies with 4253 cases and 4950 controls that evaluated the association between the *MTHFR C677T* gene polymorphism and PE. In the present studies, we detected a significant association between the *MTHFR C677T* polymorphism and PE risk in all subjects; however, when we made a stratification analysis of ethnicity, a significant association could only be found in the Asians and not in the Caucasians. This finding indicates that the potentially functional *MTHFR C677T* polymorphism may have a low penetrance role in PE susceptibility in an ethnicity-specific manner. The between-study heterogeneity existed for Asians. The sensitivity analysis showed that the Aggarwal *et al.*[53] study was the origin of the heterogeneity, and when this article was excluded, a significant association could still be found. Our results were consistent with the previously published meta-analysis by Kosmas *et al.*,[10] which showed small, but statistically significant, associations between the *MTHFR C677T* polymorphism and PE in the overall population. However, their meta-analysis did not cover Chinese databases (Chinese Biomedical Database, Chinese National Knowledge Infrastructure and Wan Fang) and did not perform subgroup analyses on the different types of study populations.

Theoretically, the *MTHFR C677T* polymorphism could cause hyperhomocysteinemia and may increase the risk of PE. However, in our meta-analysis, the *MTHFR C677T* allele increased the risk of PE in the Asians but not in the Caucasians. There are numerous possible reasons for the same polymorphism having a different role in different ethnic populations. First, an ethnic variant in the frequency of the *MTHFR C677T* mutation was demonstrated in various populations. The frequency of the 677T allele ranges from 6 to 10.3% in the African populations, from 32.2 to 44% among the Caucasian populations, and from 30.5 to 42% in the East Asian populations.[62,63] Clinical heterogeneity may also explain the discrepancy. There are many other factors that may affect Hcy levels (for example, diet, alcohol, smoking and drugs). MTHFR, folate and vitamin B12 jointly participate in the metabolism of Hcy, and therefore the influence of the *MTHFR C677T* polymorphism on Hcy levels is most prominent in subjects with low folate and vitamin B12 concentrations.[64]

The characteristics of a meta-analysis are to combine comparable studies and to increase the sample size and statistical power, thus revealing a more compelling result. However, a meta-analysis has confounding factors such as publication bias, method of sampling,

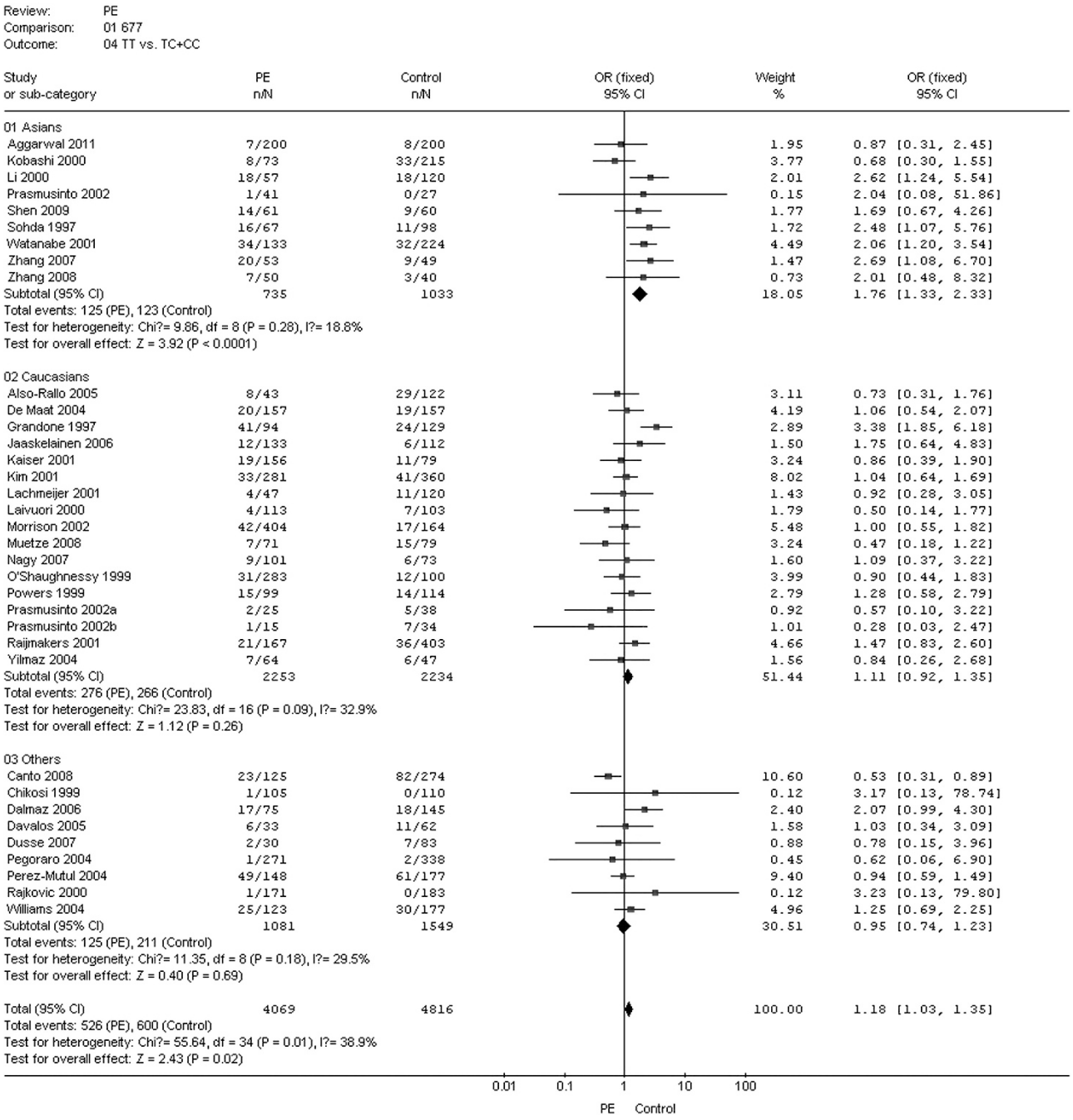

Figure 1 Forest plot for the association between *MTHFR C677T* polymorphisms and PE for the recessive genetic model (TT *vs.* TC + CC). Each study is shown by the point estimate of the OR and the 95% CI for the OR. A full color version of this figure is available at the *Hypertension Research* journal online.

varying genetic backgrounds of subjects, and varying protocols and quality of analysis. We followed the inclusion and exclusion criteria strictly to reduce the selection bias. The funnel plot and Egger's linear regression test were used to assess the publication bias. The HWE test for the distribution of the genotypes in the control groups suggested that there were no significantly different genetic backgrounds among the participants.

To some extent, a number of limitations have affected the objectivity of the conclusions and should be considered when interpreting the results. (1) Given that only published studies were included in the meta-analysis, a publication bias may have occurred, even though it was not found when performing the statistical test. (2) A language bias may have occurred because this meta-analysis only contained Chinese and English language literature. However, some relevant articles in other languages were published in specific journals but were not found in the international journals. (3) In this study, we could not obtain information from some of the studies on folate and vitamin B12 levels, which may affect the associations between the *MTHFR C677T* polymorphism and PE.

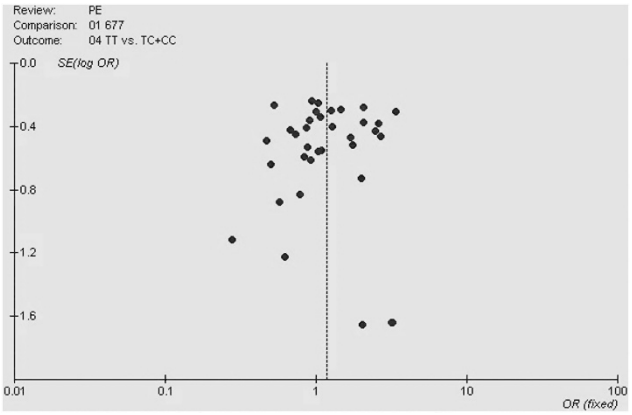

Figure 2 Funnel plot analysis to detect a publication bias for the recessive genetic model (TT *vs.* TC + CC) of the *MTHFR C677T* polymorphism in the overall analysis. Each point represents a separate study for the indicated association. OR, odds ratio. A full color version of this figure is available at the *Hypertension Research* journal online.

In conclusion, our meta-analysis suggests that the *MTHFR C677T* polymorphism may contribute to individual susceptibility to PE in the Asians; however, there is insufficient evidence to confirm this association in the Caucasians. The potentially functional *MTHFR C677T* polymorphism may have a low penetrance role in PE susceptibility in an ethnicity-specific manner. In terms of PE with multifactorial etiology, further studies or complete case–control studies are warranted, especially those stratified by ethnic backgrounds, environmental exposures or other risk factors, to clarify the possible roles of the *MTHFR C677T* polymorphism in the pathogenesis of PE.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank Dr Murphy RP for providing additional information and data on his study. We also thank the editors of this manuscript and Wei-wei Chang for providing advice for the writing and revision of the manuscript.

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