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ARTICLE

A meta-analysis of association between C677T polymorphism in the methylenetetrahydrofolate reductase gene and hypertension

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The C677T polymorphism of the methylenetetrahydrofolate reductase (MTHFR) gene was implicated to be associated with hypertension due to its role in catalyzing the formation of 5-methylenetetrahydrofolate, a co-substrate for the conversion of homocysteine to methionine. Association studies were reported in different populations; however, a great number of subsequent studies have produced contrary results, possibly reflecting inadequate statistical power. With the cumulative data in recent years in both Caucasian and Asian populations, it was necessary to carry out a comprehensive analysis of previous findings. In this meta-analysis, we combined 26 English and Chinese studies in Caucasian and Asian populations published up to November 2006 to give a new picture of the role of the C677T polymorphism in the MTHFR gene. Evidence of significant association was detected between C677T polymorphism and hypertension in both populations. Additionally, the significant association between C677T polymorphism and hypertension/hypertension-in-pregnancy suggested that this polymorphism was one independent risk factor of hypertension.
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Introduction

The development of hypertension is believed to be largely under genetic control.[1] A considerable number of genes and polymorphisms thereof have been assessed as candidate determinants of the risk for hypertension.[2] Many case–control studies have addressed in particular the putative role of a C-to-T mutation at nucleotide 677 in the methylenetetrahydrofolate reductase (MTHFR) gene. MTHFR catalyzes the formation of 5-methylenetetrahydrofolate, a co-substrate for the conversion of homocysteine to methionine.[3–5] The T allele has been associated in its homozygous form with elevated homocysteine through the creation of a thermolabile enzyme isoform with reduced activity,[6] and a high plasma concentration of homocysteine may predispose to atherosclerosis by injuring the vascular endothelium, which results in hypertension.[7] The genotype frequencies of the polymorphism are C/C, 0.583; C/T, 0.35; T/T, 0.067 in Europeans and C/C, 0.267; C/T, 0.444; T/T, 0.289 in Chinese (www.hapmap.org). A number of population studies provided 'positive' and 'negative' results in both hypertension and hypertension-in-pregnancy. However, the results of the genetic association studies on the role of the C677T MTHFR polymorphism in hypertension have generated

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considerable controversy.[8,9] Given the accumulation of data, we decided to perform a formal meta-analysis to reconcile the conflicting findings.

Methods

Literature search

The literature included in the analysis was selected using PubMed and CNKI (Chinese) with keywords 'methylenetetrahydrofolate reductase' or 'MTHFR' and 'hypertension'. The search was complemented with a perusal of the bibliographies of retrieved papers and review articles. Eligible studies had to meet all of the following criteria: (1) they were published in a peer-reviewed journal, (2) they contained independent data, (3) they presented sufficient data to calculate the odds ratio (OR) with a confidence interval and a *P*-value, (4) they were association studies, (5) they described the relevant genotyping primers, machines and protocols or provided reference to them, (6) they diagnosed patients according to the criteria of SBP $\geq$ 140 or DBP $\geq$ 90 and (7) they used healthy individuals as controls. Authors were contacted in cases where there were queries regarding their studies.

Statistical analyses

Data from the case–control studies were used to construct a 2×2 table in which subjects were classified by diagnostic category and type of allele. Cochran's χ^2-based Q statistic test was performed in order to assess possible heterogeneity in the combined studies. Where heterogeneity existed, the random effects model, which yields wider confidence intervals (CIs), was adopted; otherwise both the fixed effects and random effects models were deemed appropriate. A test for funnel plot asymmetry, described by Egger *et al*,[10] was used to assess evidence for publication bias. ORs were pooled using the DerSimonian and Laird[11] method and 95% CIs were constructed using Woolf's[12] method. The significance of the overall OR was determined by the *Z*-test. The analysis was conducted on Comprehensive Meta Analysis software (Version1.0.23, BIOSTAT, Englewood, NJ, USA).

Results

The combined search yielded at least 167 references. After discarding overlapping references and those that clearly did not meet the criteria, 26 studies were identified[13–39] for recruitment. Of these, 13 were case–control studies on the association of C667T polymorphism in the MTHFR gene and hypertension in pregnancy. All studies were published between 1998 and 2006. The descriptive characteristics of all the 26 studies included in our meta-analytic study were coded using the following variables: the first author (year of publication), the ancestry of the sample, the year of

Table 1 Characteristics of the included studies

First author (year)	*Ancestry*	*Enrollment*	*Cases/controls*	*Diagnostic standard*
Hypertension				
Yukiko Nakata (1998)	Japanese	1992–1995	173/184	SBP $\geq$ 160, DBP $\geq$ 95
Siyan Zhan (2000)	Chinese	1997–2001	127/170	SBP $\geq$ 140, DBP $\geq$ 90
Petr Benes (2001)	Caucasian	1996–2000	193/209	SBP $\geq$ 140, DBP $\geq$ 90
Lin Wang (2002)	Chinese	2000–2001	105/46	SBP $\geq$ 140, DBP $\geq$ 90
Xiaonan Sun (2003)	Chinese	No data	55/46	SBP $\geq$ 140, DBP $\geq$ 90
Stephanie HEUX (2004)	Caucasian	No data	247/249	SBP $\geq$ 140, DBP $\geq$ 90
Jianwei Liu (2004)	Chinese	No data	159/100	SBP $\geq$ 140, DBP $\geq$ 90
Leszek Tylicki (2005)	Austrian/Polish	2002–2003	90/90	SBP $\geq$ 140, DBP $\geq$ 90
Rile Hu (2006)	Mongolian	No data	110/115	SBP $\geq$ 140, DBP $\geq$ 90
Htay Lwin (2006)	Japanese	1999–2000	116/219	SBP $\geq$ 140, DBP $\geq$ 90
B Nagy (2006)	Hungarian	2000–2005	101/73	SBP $\geq$ 160, DBP $\geq$ 90
Xiuxiu Li (2006)	Chinese	2003	72/30	No data
Hypertension in pregnancy				
Robert W Powers (1999)	Caucasian	No data	123/114	SBP $\geq$ 140, DBP $\geq$ 90
Elvira Grandone (1999)	Caucasian	No data	139/216	SBP $\geq$ 140, DBP $\geq$ 90
Keshen Li (2000)	Chinese	No data	62/90	SBP $\geq$ 140, DBP $\geq$ 90
Gen Kobashi (2000)	Japanese	No data	174/215	SBP $\geq$ 140, DBP $\geq$ 90
Shuqing Wei (2001)	Chinese	1999–2000	42/36	SBP $\geq$ 140, DBP $\geq$ 90
Feng Fu (2003)	Chinese	2001–2002	102/100	SBP $\geq$ 140, DBP $\geq$ 90
Hulya Yilmaz (2004)	Turkish	No data	64/47	SBP $\geq$ 140, DBP $\geq$ 90
Haiyan Wang (2004)	Chinese	2000–2003	99/54	SBP $\geq$ 140, DBP $\geq$ 90
Dongwei Mao (2004)	Chinese	1998–2002	100/100	SBP $\geq$ 140, DBP $\geq$ 90
Geng Tian (2005)	Chinese	2003–2004	61/56	no data
Sonia Hernandez-Diaz (2005)	Caucasian	1993–1998	54/100	SBP $\geq$ 140, DBP $\geq$ 90
Sumin Wang (2006)	Chinese	2002–2004	54/125	SBP $\geq$ 140, DBP $\geq$ 90
Gen Kobashi (2006)	Japanese	1993–1998	100/100	SBP $\geq$ 140, DBP $\geq$ 90

sample enrolment, the size of design and the diagnostic standard of each study (Table 1).

MTHFR C677T TT genotype and risk for hypertension

Global statistical results Of the 25 case–control association studies (data not sufficient in one study[37]) of the C677T genotype TT of the MTHFR gene and hypertension, only five[15,19,24,25,34]showed a statistically significant difference in allele frequencies between hypertensive and control subjects (see Figure 1a). The pooled OR derived from 2814 hypertensive and 3099 control subjects in 25 recruited studies was statistically significant (see Table 2). Homogeneity analysis for the ORs from the 25 studies of the genotype TT suggested that there was statistically significant evidence for heterogeneity of the ORs among the groups of genotype TT studies (see Table 2). There was no significant evidence of publication bias detected in the total studies (Table 2 and Figure 2a).

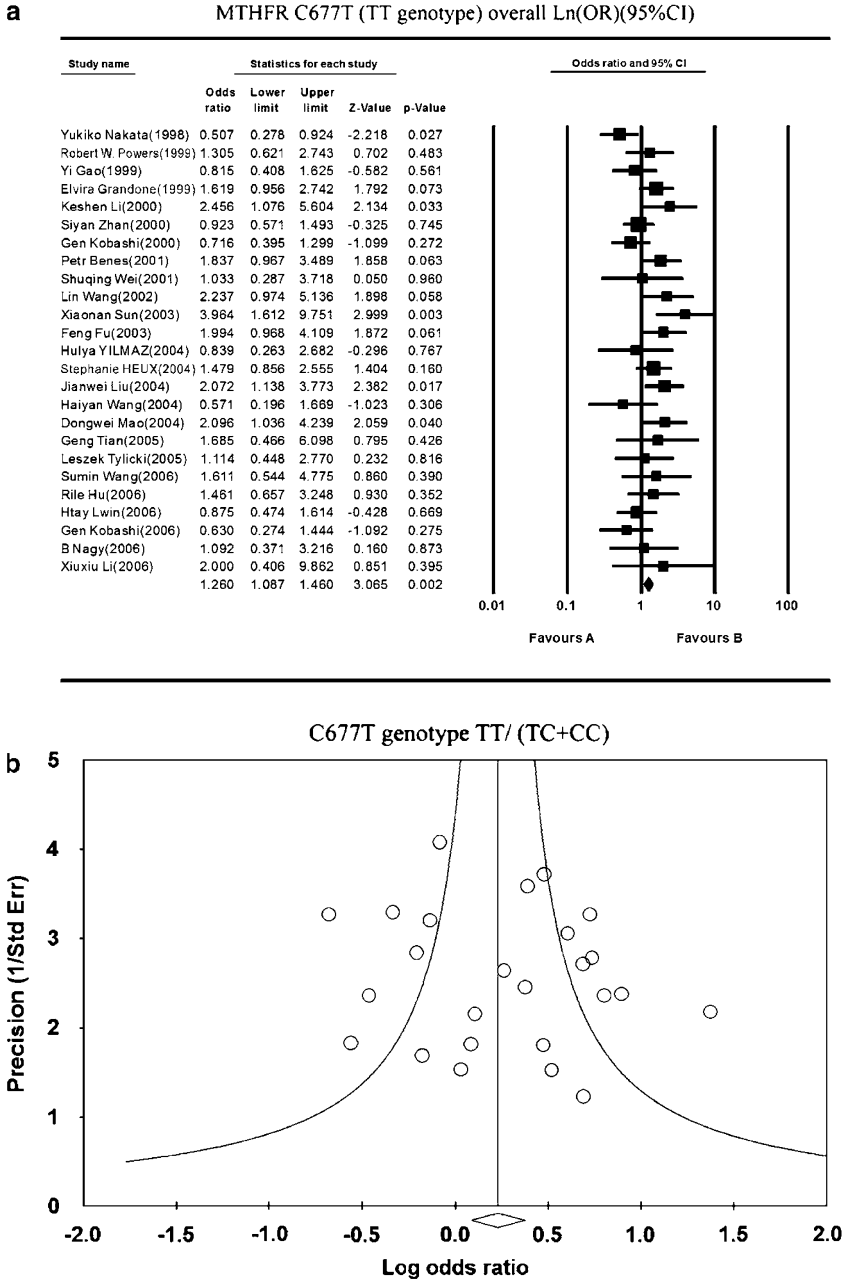

Figure 1 (**a**, **b**) Forest plots of ln (OR) with 95% CI for studies with hypertension. Black squares indicate the ln (OR), with the size of the square inversely proportional to its variance, and horizontal lines represent the 95% CIs. The pooled results are indicated by the unshaded black diamond.

Table 2 Basic statistical data of MTHFR polymorphism and risk for hypertension

	TT/(TC+CC)		*(TT+TC)/CC*	
Group	*Heterogeneity*	*Pooled OR*	*Heterogeneity*	*Pooled OR*
HP	$Q=16.075$, df = 11, $P=0.138$	OR = 1.282, 95% CI = 0.959–1.714, $z=1.679$, $P=0.093$, random	$Q=24.060$, df = 11, $P=0.012$	OR = 1.661, 95% CI = 1.387–1.989, $z=5.515$, $P<0.001$, random
H	$Q=26.25$, df = 12, $P=0.010$	OR = 1.235, 95% CI = 1.020–1.497, $z=2.158$, $P=0.031$, fixed	$Q=13.833$, df = 12, $P=0.312$	OR = 1.165, 95% CI = 1.006–1.350, $z=2.034$, $P=0.042$, fixed
Asian	$Q=38.866$, df = 17, $P=0.002$	OR = 1.189, 95% CI = 0.996–1.419, $z=1.913$, $P=0.056$, random	$Q=42.391$, df = 17, $P=0.001$	OR = 1.403, 95% CI = 1.218–1.617, $z=4.681$, $P<0.001$, random
Caucasian	$Q=2.206$, df = 6, $P=0.900$	OR = 1.438, 95% CI = 1.101–1.879, $z=2.663$, $P=0.008$, fixed	$Q=3.368$, df = 6, $P=0.761$	OR = 1.239, 95% CI = 1.022–1.502, $z=2.186$, $P=0.029$, fixed
Total	$Q=42.427$, df = 24, $P=0.012$	OR = 1.260, 95% CI = 1.087–1.460, $z=3.065$, $P=0.002$, random	$Q=46.792$, df = 24, $P=0.004$	OR = 1.416, 95% CI = 1.200–1.670, $z=4.118$, $P<0.001$, random
Total publication bias	$a=0.614$ (95% CI = −1.375–2.604), $t=0.639$, df = 23, $P=0.529$		$a=2.773$ (95% CI = 0.853–4.693), $t=2.988$, df = 23, $P=0.006$	

HP, hypertension in pregnancy; H, hypertension; Asian, Asian studies; Caucasian, Caucasian studies. Chinese, Japanese and Mongolian studies in this analysis were defined as Asian and the rest as Caucasian.

Significance tests of ORs between the groups

Hypertension-in-pregnancy studies and hypertension studies ($z=3.065$, $P=0.002$) No significant heterogeneity was observed after stratification of the group of ORs by sample constitution in 12 hypertension-in-pregnancy studies, but it remained in 13 hypertension studies. The pooled ORs for the studies of the genotype TT derived from hypertension samples showed statistical significance but were not present among hypertension-in-pregnancy studies (Table 2).

Studies in different populations ($z=3.065$, $P=0.002$) No significant heterogeneity was observed after stratification of the group of ORs by sample constitution in seven studies in Caucasians, but it remained in 18 studies in Asians. The pooled ORs for the studies of the genotype TT derived from western studies showed statistical significance but were not present among studies in Asians (Table 2).

MTHFR C677T T carriers and risk for hypertension

Global statistical results Of the 25 case–control association studies of the C677T allele T carriers of the MTHFR gene and hypertension, six[9,15,16,19,24,26] showed a statistically significant difference in allele frequencies between hypertensive and control subjects (data not sufficient in one study[39]) (see Figure 1b). The pooled OR derived from 2814 hypertensive and 3099 control subjects was statistically significant (see Table 2). Homogeneity analysis for the ORs from the 25 studies of the allele T carriers suggested that there was statistical significance evidence for heterogeneity of the ORs among the groups of allele T carriers' studies (see Table 2). There was significant evidence of publication bias detected in the total studies (Table 2 and Figure 2b).

Significance tests of ORs between the groups

Hypertension-in-pregnancy studies and hypertension studies ($z=5.065$, $P<0.001$) No significant heterogeneity was observed after stratification of the group of ORs by sample constitution in H studies, but it remained in HP studies. The pooled ORs for the studies of the allele T derived from both H and HP studies showed statistical significance (Table 2).

Asian studies and Caucasian studies ($z=5.065$, $P<0.001$) No significant heterogeneity was observed after stratification of the group of ORs by sample constitution in seven western studies, but it remained in 18 Asian studies. The pooled ORs for the studies of the allele T derived from Caucasian and Asian studies showed statistical significance (Table 2).

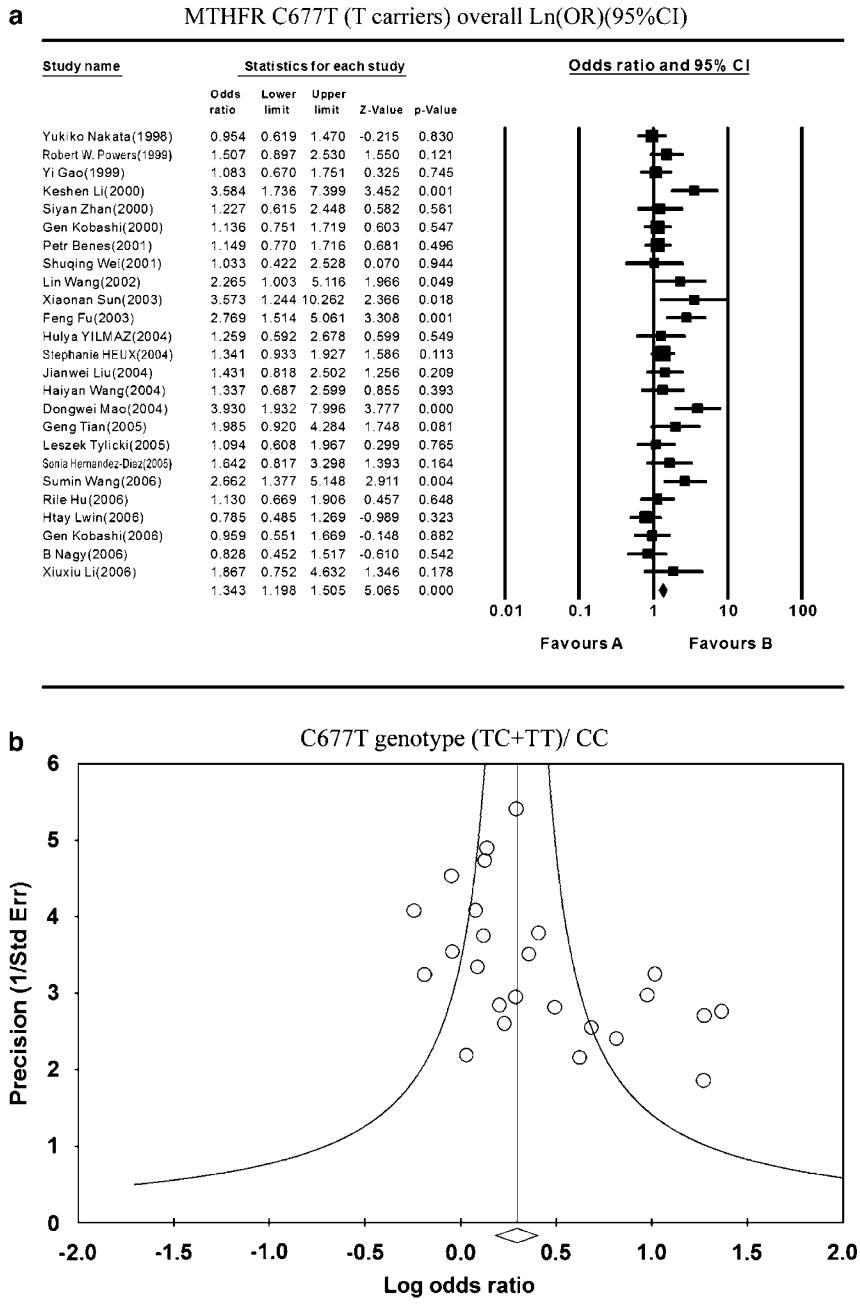

Figure 2 (**a**, **b**) Egger's funnel plots of publication bias analysis for studies with hypertension. The larger deviation from funnel curve of each study means the more pronounced asymmetry. Results from small studies will scatter widely at the bottom of the graph, with the spread narrowing among larger studies.

Discussion

Meta-analysis is a powerful tool for analyzing cumulative data of studies where the individual sample sizes are small and the statistical power low. This meta-analysis has included data for the C677T MTHFR polymorphism from over 2800 subjects who were hypertensive, along with a similar number of normotensive controls. We found an association of this polymorphism with hypertension and hypertension-in-pregnancy, but there was large and statistically significant heterogeneity in the results of different studies. This necessitated a number of subgroup and bias analyses that yielded some interesting findings. Significant heterogeneity for ORs was detected in eastern studies after subgroup analysis, which suggested that the analytic results from eastern samples may not be reliable.

Time lag bias and publication bias may have affected these results. In time lag bias,[40] studies with 'negative' results take longer time to be published, while enthusiastic results are published much more quickly. In publication bias,[41,42] small studies with 'negative' results are never published, while equally small studies with similar quality but 'positive' results would appear in the literature. We examined these possibilities and found that, indeed, 'positive' studies were reported more in T allele carriers' studies (especially in eastern studies), which could explain the existence of bias. Other phenomena of bias were also observed in the subgroup analysis, which we ascribed to insufficiency for the studies after grouping.

Our study has advantages compared with the meta-analysis previously reported.[43] We inspected not only hypertension but also hypertension-in-pregnancy and the accordant results suggested that the association between this polymorphism and vascular disease that increases the risk both for hypertension and hypertension in pregnancy may be seen only in the setting of hyperhomocysteinemia.[44–46] Elevated levels of homocysteine may be correlated with hypertension and hypertension in pregnancy.[47] Dietary parameters may be acting as effect modifiers in this genetic association and may cause heterogeneity in the observed genetic effects across studies.[43]

In all, our meta-analysis suggested significance association between MTHFR C677T polymorphism and hypertension. The heterogeneity of the ORs we have identified among the studies of eastern populations is a real problem. Further clarification using enlarged sample sizes and additional family-based TDT studies is essential. Our meta-analysis of the 26 available population-based case–control studies points to inadequate statistical power, differences in geographic or ethnic background and potential gene–environment interactions all contributing to difficulty in interpreting the results in previous studies.

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