

Original Article

Is methylenetetrahydrofolate reductase C677T polymorphism associated with hyperuricemia?

Shujun Fan^{1*}, Boyi Yang^{1*}, Xueyuan Zhi¹, Yanxun Wang², Jian Wei³, Guifan Sun¹

¹Research Center of Environment and Non-Communicable Disease, School of Public Health, China Medical University, Shenyang, China; ²Division of Molecular Preventive Medicine, Shanghai Institute of Targeted Therapy and Molecular Medicine, Shanghai, China; ³Tianjin Dagang Oil Field General Hospital, Tianjin, China. *Equal contributors.

Received February 27, 2016; Accepted May 22, 2016; Epub July 1, 2016; Published July 15, 2016

Abstract: Several epidemiological studies have examined the relationship of the methylenetetrahydrofolate reductase (*MTHFR*) C677T polymorphism with hyperuricemia. However, the results were controversial. We therefore conducted a large cross-sectional study (2,219 subjects) and an updated meta-analysis by combining previous studies (9,502 subjects) to provide more empirical evidence for the association. And we assessed the strength of the association using Venice criteria, which is a recently developed epidemiological grading system. In our cross-sectional study, the *MTHFR* C677T polymorphism was not associated with hyperuricemia in any genetic model. The following overall meta-analysis showed a significant association; however, there existed high between-study heterogeneity and obvious publication bias. Furthermore, subgroup analyses showed that there was no significant association in studies with high quality (large sample size and more accurate genotyping method). Cumulative meta-analysis and sensitivity analysis also indicated that the meta-analytical results were not statistically robust. According to the Venice criteria, the cumulative evidence was graded as having very weak credibility. All these results suggest that the significant association between the *MTHFR* C677T polymorphism and hyperuricemia may be questionable. Further well-designed studies with larger sample sizes are greatly needed to confirm or refute our findings.

Keywords: Hyperuricemia, *MTHFR* C677T, polymorphism, meta-analysis

Introduction

Hyperuricemia has long been linked to the development of gouty arthritis in humans. Recently, accumulating evidence shows that hyperuricemia also is a risk factor for metabolic syndrome and cardiovascular diseases [1-4]. The prevalence of hyperuricemia has been estimated as 13.7% in China, and showed geographical and sex variations [5]. Many factors are known to influence serum uric acid levels. Among them, genetic determinants are estimated to explain about 25-63% of the variation of uric acid levels [6]. Thus, identification of hyperuricemia susceptibility genes will help clarify the pathogenesis of the disease and provide new therapeutic and preventive strategies.

Over the past decade, a large number of genetic studies have been performed to decipher the

genetic architecture of hyperuricemia, and hundreds of genes and polymorphisms have been hypothesized to be involved in the pathogenesis of hyperuricemia [7-10]. Of these, Methylenetetrahydrofolate reductase (*MTHFR*) C677T polymorphism was considered as a potential candidate [11-20]. The *MTHFR* is an important enzyme in homocysteine metabolic pathway that irreversibly catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which serves as the carbon donor in the remethylation of homocysteine to methionine. The most common mutation in the *MTHFR* gene is the C677T polymorphism, which has been confirmed to affect the enzyme activity and ultimately lead to accumulation of homocysteine [21]. Several previous studies have reported a significant relationship between homocysteine and uric acid levels [22-24]. The mechanism underlying this association remains unclear; however, some investigators have pos-

tulated that elevated homocysteine levels might reflect the consequence of renovascular atherosclerosis and the complication of systemic vascular disease, which is associated with decreased renal clearance of uric acid and elevated serum uric acid concentrations [22, 25]. Furthermore, adenosine originating from S-adenosyl-L-homocysteine, a precursor of homocysteine, could represent a link between the metabolic pathway of homocysteine and uric acid [24].

A number of epidemiological studies have been conducted to examine the relationship of the *MTHFR* C677T polymorphism with the risk of hyperuricemia [11-20]. However, the results were controversial. A cross-sectional study by Zuo *et al.* showed that the *MTHFR* 677T allele carriers had an increased hyperuricemia risk than the 677C allele carriers among elderly Japanese men [11]. Hong *et al.* reported that the *MTHFR* 677TT genotype was significantly more frequent in subjects with high uric acid levels in Korean population [12]. Golbahar and coworkers observed that the *MTHFR* C677T polymorphism was a strong predictor of uric acid in males and in females among Iranians [15]. However, two recent large studies in Japanese and Chinese populations reported the association was not significant [19, 20]. Non-replication of genetic studies is common in complexity chronic diseases, and the genetic-disease association is usually geographical and ethnic-dependent, which may be caused by population heterogeneity in genetic background and environmental exposures. Thus exploration of gene-disease association in each area (country) or ethnic group is essential. Chinese researchers have conducted several case-control studies claiming or refuting the relationship between the *MTHFR* C677T polymorphism and hyperuricemia [13, 14, 16-18, 20]. However, these studies were limited by small sample size and the results were controversial. In addition, the incidence of hyperuricemia and its related chronic diseases is high in China, especially in northern regions, as did prevalence of hyperhomocysteinemia and the 677T allele observed in our previous studies [26, 27]. Exploration of the relationship between the *MTHFR* C677T polymorphism and hyperuricemia is therefore necessary and of great importance. We conducted a large sample size cross-sectional study in a northern Chinese Han population to investigate the

association. Furthermore, to set the results of our study in the context of other previous findings and to provide more comprehensive evidence for the association, we carried out an updated meta-analysis combining the present study and previous published studies.

Materials and methods

Cross-sectional study

Study subjects: The study subjects were selected from 2,232 residents aged 21 years or older in Tianjin Municipality, China, who took regular health examinations at the physical examination center of Dagang Oil Field General Hospital. All participants were of the same ethnic background (Han nationality) and were not first- or second-degree relatives. We excluded three participants without blood pressure information and ten participants with gout or renal failure ($\geq 132 \mu\text{mol/L}$ of serum creatinine). Finally, the remaining 2,219 subjects (1,477 males and 742 females) were included. This study was approved by the ethics review committee of the China Medical University (Shenyang, China; Identification Code: CMU-62073024; 15 July 2008) and was conducted in accordance with the Helsinki Declaration. All participants gave written informed consent prior to study entry.

Data collection and definition: Body weight and height were measured using a standard scale with light clothing and barefoot. Body mass index (BMI) was computed as weight in kilograms divided by the square of height in meters (kg/m^2). Blood pressure was measured while subjects were in the sitting position after 15 min of rest. The average of three measurements was recorded. The concentrations of creatinine, uric acid, total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and fasting blood glucose (FBG) were determined by enzymatic method using a Hitachi Autoanalyzer (Type 7170A; Hitachi Ltd., Tokyo, Japan). Hyperuricemia was defined as uric acid level $\geq 360 \mu\text{mol/L}$ for females and $\geq 420 \mu\text{mol/L}$ for males [28].

Genotyping analysis

Genomic DNA was extracted from buccal samples using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA). The genotypes of

MTHFR C677T polymorphism and hyperuricemia

Table 1. Basic characteristic of the participants in the study

Characteristics	Total	Males	Females	P
Number of subjects	2219	1477	742	-
Age (years)	46.83 ± 9.85	47.43 ± 10.04	45.64 ± 9.34	< 0.001
BMI (kg/m ²)	25.03 ± 3.54	25.80 ± 3.37	23.51 ± 3.38	< 0.001
Creatinine (μmol/L)	75.41 ± 12.72	79.88 ± 11.47	66.51 ± 10.15	< 0.001
Uric acid (μmol/L)	324.13 ± 92.96	358.35 ± 86.44	256.01 ± 63.29	< 0.001
TC (mmol/L)	4.98 ± 0.99	5.00 ± 0.98	4.92 ± 1.00	0.068
TG (mmol/L)	1.35 ± 1.17	1.51 ± 1.25	1.02 ± 0.91	< 0.001
HDL-C (mmol/L)	1.20 ± 0.38	1.45 ± 0.37	1.32 ± 0.37	< 0.001
LDL-C (mmol/L)	2.91 ± 0.98	2.97 ± 1.00	2.81 ± 0.94	< 0.001
FBG (mmol/L)	5.30 ± 1.18	5.43 ± 1.26	5.06 ± 0.95	< 0.001
SBP (mmHg)	130.49 ± 19.46	133.70 ± 18.83	124.11 ± 19.13	< 0.001
DBP (mmHg)	82.43 ± 12.99	85.10 ± 13.09	77.11 ± 11.01	< 0.001

BMI, body mass index; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FBG, fasting blood glucose; SBP, systolic blood pressure; DBP, diastolic blood pressure.

the *MTHFR* C677T were determined by a TaqMan assay, which has been detailed in our previous paper [27].

Statistical analysis

Differences in the distribution of baseline characteristics (age, BMI, creatinine, uric acid, TC, TG, HDL-C, LDL-C, FBG, SBP and DBP levels) between females and males were tested with the student's t-test. The *MTHFR* C677T allele and genotype frequencies in the participants were calculated by direct counting. Chi-square test was performed to identify departures from the Hardy-Weinberg equilibrium (HWE), and to compare the differences between hyperuricemia group and normal uric acid level group with respect to allelic and genotypic frequencies. The unconditional logistic regression analysis was used to estimate the effects of the *MTHFR* C677T polymorphism on hyperuricemia risk under five genetic models (homozygous codominant, heterozygous codominant, dominant, recessive and allelic models), without and with adjustment for potential risk factors. Odds ratio (OR) with 95% confidence index (CI) was calculated to estimate the relative risk of hyperuricemia associated with the different genotypes and alleles. Two-sided *P* value below 0.05 was considered to be statistically significant. These analyses were conducted using SAS Version 9.2 (SAS Institute, Cary, NC, USA).

Meta-analysis

We performed a systematic literature search in three English (PubMed, Embase and Web of

Science) and four Chinese databases (China National Knowledge Infrastructure (CNKI), Wanfang, Chongqing VIP Chinese Science and Technology Periodical Database (VIP) and China Biological Medicine Database (CBM)) for studies exploring the relationship of the *MTHFR* C677T polymorphism with hyperuricemia. The strategy was based on combinations of the following search items: hyperuricemia, high uric acid, uric acid, *MTHFR*, methylenetetrahydrofolate reductase, allele, gene, genotype, variant, variation and polymorphism. The reference lists were hand-searched to find potentially eligible studies. Studies included in the meta-analysis had to meet the following criteria: (1) cross-sectional or cohort studies; (2) evaluating the association of the *MTHFR* C677T polymorphism with hyperuricemia risk, and (3) providing sufficient data for calculating the OR with 95% CI.

Two investigators independently reviewed and extracted the following items from each included study: first author's name, year of publication, country and ethnicity of study population, study design, genotyping method, diagnostic criteria of hyperuricemia, mean age, and number of alleles and genotypes in both hyperuricemia and normal groups. Discrepancy was resolved by discussion between the two investigators.

The association of the *MTHFR* C677T polymorphism with hyperuricemia risk was assessed by calculating pooled ORs with the corresponding 95% CIs under the homozygous codominant,

MTHFR C677T polymorphism and hyperuricemia

Table 2. Association of the *MTHFR* C677T polymorphism with hyperuricemia risk

Polymorphism	Hyperuricemia (n = 384)	Normal (n = 1835)	Crude OR (95% CI)	P	Adjusted OR ^a (95% CI)	P
Codominant						
CC	84 (21.88)	402 (21.91)	1.00	-	1.00	-
CT	201 (52.34)	858 (46.76)	1.12 (0.85-1.49)	0.425	0.96 (0.71-1.31)	0.814
TT	99 (25.78)	575 (31.33)	0.82 (0.60-1.13)	0.232	0.71 (0.50-1.01)	0.058
Dominant						
CC	84 (21.88)	402 (21.91)	1.00	-	1.00	-
CT + TT	300 (78.12)	1433 (78.09)	1.00 (0.77-1.31)	0.989	0.86 (0.64-1.16)	0.862
Recessive						
CC + CT	285 (74.22)	1260 (68.67)	1.00	-	1.00	-
TT	99 (25.78)	575 (31.33)	0.76 (0.59-0.98)	0.032	0.73 (0.56-0.96)	0.026
Allelic						
C	369 (48.05)	2061 (50.65)	1.00	-	-	-
T	399 (51.95)	2008 (49.35)	0.93 (0.77-1.11)	0.397	-	-

OR, odds ratio; CI, confidence index. ^aAdjusted by age, sex, creatinine, triglycerides, total cholesterol, diastolic blood pressure and body mass index.

heterozygous codominant, dominant, recessive and allelic models. The heterogeneity was considered statistically significant if $P < 0.05$ for Cochran's chi-square based Q-test or $I^2 > 50\%$ for I^2 statistic [29, 30]. If the between-study heterogeneity was statistically significant, the random effects model was used; otherwise, the fixed effects model was used [31]. The HWE for each study was tested again. Subgroup analyses by ethnicity, study design, source of control, genotyping method, sample size and gender. Meta-regression analysis was employed to explore the sources of heterogeneity [32]. Cumulative meta-analysis was performed by date of publication exploring the dynamic trends as studies accumulated over time [33]. Sensitivity analyses excluding each study were used to assess the stability of the results [33]. Publication bias was investigated by the funnel plot and Egger's regression test [34]. We also used the Venice criteria to assess the strength of the cumulative evidence [35]. All meta-analysis was performed by the STATA package Version 11.0 program (StataCorp, College Station, TX, USA), and P value below 0.05 was considered to be statistically significant.

Results

Population characteristics in our cross-sectional study

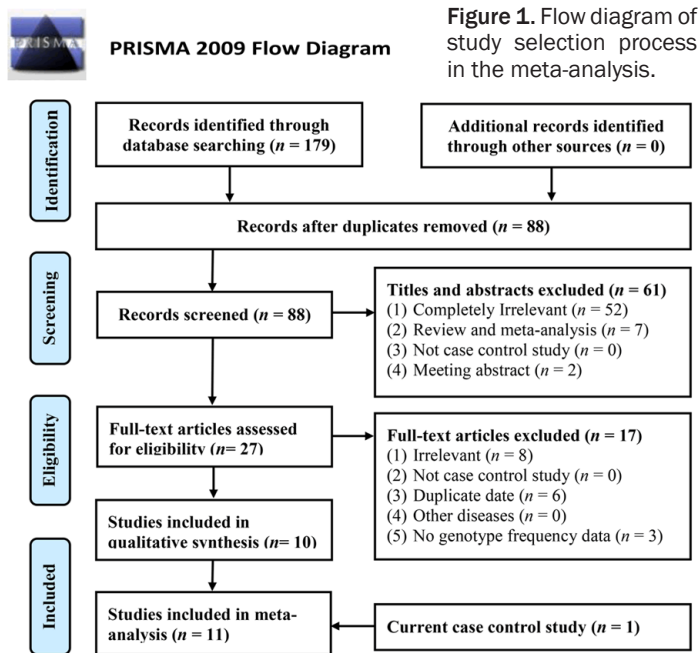
Table 1 summarizes the demographic and clinical characteristics of 1,477 males and 742

females in the study. The mean age of the participants was 46.83 ± 9.85 years. The prevalence of hyperuricemia was 17.31% (males vs. females was 22.88% vs. 6.20%). Compared with females, males had significantly higher hyperuricemia prevalence, BMI, creatinine, uric acid, TG, HDL-C, LDL-C, FBG, SBP and DBP levels (All $P < 0.05$).

Genotype distribution and association analysis in our cross-sectional study

Table 2 presents the genotypic and allelic frequencies of the *MTHFR* C677T polymorphism. The genotype distribution of the polymorphism among the study population was in consistent with HWE ($P = 0.069$). The 677T allele and 677TT genotype frequencies were not significantly different between hyperuricemia and normal uric acid levels groups ($P = 0.071$ and 0.186, respectively). Furthermore, we used the unconditional logistic regression analysis to test the associations of the *MTHFR* C677T polymorphism with hyperuricemia risk under five genetic models. The results indicated that there was no association between the *MTHFR* C677T polymorphism and hyperuricemia under heterozygous codominant (adjusted OR = 0.96, 95% CI = 0.71-1.31), homozygous codominant (adjusted OR = 0.71, 95% CI = 0.50-1.01), dominant (adjusted OR = 0.86, 95% CI = 0.64-1.16) and allelic models (unadjusted OR = 0.93, 95% CI = 0.77-1.11). When stratified by gender, no significant association was detected for either

MTHFR C677T polymorphism and hyperuricemia



males or females under any of the tested genetic models (Tables S1 and S2).

Meta-analysis results

Figure 1 details the process of study selection. A total of 11 studies [11-20] (including the current study) comprising 2,401 individuals with hyperuricemia and 7,101 individuals with normal uric acid levels were included in the meta-analysis. The detailed characteristics and genotype distributions of each included study are presented in Table S3 and Table 3, respectively.

The results of this meta-analysis indicated that the *MTHFR* C677T polymorphism was significantly associated with the risk of hyperuricemia in all genetic models (dominant: OR = 1.94, 95% CI = 1.39-2.70; recessive: OR = 1.43, 95% CI = 1.07-1.90; homozygous codominant: OR = 2.07, 95% CI = 1.39-3.09; heterozygous codominant: OR = 1.81, 95% CI = 1.34-2.45; allelic: OR = 1.59, 95% CI = 1.25-2.02) (Figures S1, S2, S3, S4, S5). According to the principle of genetic model selection, the dominant model was used to conduct the subsequent analyses [36]. After exclusion of HWE-violating studies, the corresponding pooled ORs were not changed appreciably. Subgroup analysis by ethnicity showed that the relationship of the *MTHFR* C677T polymorphism and hyperuricemia

was stronger in Caucasians (OR = 3.24, 95% CI = 2.26-4.65) than in East Asians (OR = 1.78, 95% CI = 1.31-2.41). Additionally, in stratified analyses according to study design, source of control, genotyping method, sample size and gender, the *MTHFR* C677T polymorphism was significantly associated with hyperuricemia in all the subgroups with the exception of "others" genotyping method studies and large sample size studies (Table 4). The results of meta-regression analyses indicated that sample size ($P = 0.054$) and genotyping methods ($P < 0.001$) might be the sources of heterogeneity, while ethnicity ($P = 0.315$), study design ($P = 0.453$), source of control ($P = 0.991$), and gender ($P = 0.162$) were not significantly associated with the detected heterogeneity.

Subsequently, we performed cumulative meta-analysis and sensitivity analysis to evaluate the stability and liability of the results. In cumulative meta-analysis, we found that before 2013 the significant ORs increased with a narrowing of the 95% CIs as information accumulated by year. However, the addition of three later studies reduced the pooled ORs slightly and widened its corresponding 95% CIs (Figure S6). In sensitivity analysis, exclusion of any study did not materially affect the magnitude and significance of the association, with the exception of studies by Golbahar *et al.* [15] and Hinohara *et al.* [19]. As shown in Figure S7, the summarized association remained significant but the pooled ORs reduced to 1.24 (1.10-1.38) and increased to 1.67 (1.45-1.92) after omitting studies by Golbahar *et al.* [15] and Hinohara *et al.* [19], respectively.

The shape of the funnel plots appeared to be asymmetry under the dominant genetic model, suggesting the presence of publication bias (Figure S8). The results of Egger's test also showed that there was strong statistical evidence of publication bias ($P = 0.002$). According to the Venice interim criteria, the cumulative evidence for the significant association observed in the meta-analysis was weak because of high heterogeneity and publication bias.

MTHFR C677T polymorphism and hyperuricemia

Table 3. Genotypic and allelic distributions of *MTHFR* C677T polymorphism used in the meta-analysis

Study First Author (Year) [Ref]	Genotype distribution						Allele frequency				
	Hyperuricemia			Normal			Hyperuricemia		Normal		HWE
	CC	CT	TT	CC	CT	TT	C	T	C	T	P
Zuo <i>et al.</i> (2000) [11]	15	30	13	96	92	25	60	56	284	142	0.631
Hong <i>et al.</i> (2004) [12]	20	49	18	97	113	30	89	85	307	173	0.503
Lili Ding (2005) [13]	19	54	20	29	45	10	92	94	103	65	0.083
Shi <i>et al.</i> (2006) [14]	27	42	21	40	38	13	96	84	118	64	0.249
Golbahar <i>et al.</i> (2007) [15]	87	125	19	190	85	12	299	163	465	109	0.285
Wang <i>et al.</i> (2007) [16]	35	68	50	42	38	20	138	168	122	78	0.010
Mai <i>et al.</i> (2008) [17]	16	19	12	33	7	2	51	43	73	11	0.004
Zhang <i>et al.</i> (2008) [18]	23	40	25	26	17	9	86	90	69	35	0.037
Hinohara <i>et al.</i> (2013) [19]	257	350	112	1316	1797	593	864	574	4429	2983	0.063
Yuyang Pan (2014) [20]	113	231	107	123	209	119	457	445	455	447	0.466
Present study	84	201	99	402	858	575	369	399	1662	2008	0.069

HWE, Hardy-Weinberg equilibrium.

Table 4. Stratified analysis of the associations of the *MTHFR* C677T polymorphism with hyperuricemia under dominant model

Subgroup	N	Sample size	OR (95% CI)	P_z	I^2 (%)	P_h
Overall	11	9502	1.94 (1.39-2.70)	< 0.001	85.9	< 0.001
HWE	8	9020	1.63 (1.16-2.30)	0.005	85.7	< 0.001
Ethnic						
Caucasian	1	518	3.24 (2.26-4.65)	< 0.001	-	-
East Asian	10	8984	1.78 (1.31-2.41)	< 0.001	80.3	< 0.001
Study design						
Cross sectional	5	7760	1.70 (1.04-2.80)	0.036	91.1	< 0.001
Case-control	6	1742	2.23 (1.38-3.59)	0.001	75.1	0.001
Source of control						
Population based	5	5722	1.96 (1.08-3.56)	0.027	90.7	< 0.001
Hospital based	6	3780	1.95 (1.22-3.10)	0.005	82.1	< 0.001
Genotyping method						
PCR-RFLP	7	1779	2.73 (2.21-3.38)	< 0.001	16.2	0.306
Others	4	7723	1.04 (0.92-1.18)	0.527	34.2	0.207
Sample size						
Large study	4	8064	1.35 (0.86-2.12)	0.188	91.6	< 0.001
Small study	7	1438	2.45 (1.92-3.11)	< 0.001	2.7	0.405
Sex						
Males	8	6305	1.78 (1.22-2.59)	0.003	84.9	< 0.001
Female	4	2623	1.71 (0.98-2.98)	0.061	60.9	0.053

OR, odds ratio; CI, confidence interval; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; HWE, Hardy-Weinberg equilibrium. P_z , P value for association test; P_h , P value for heterogeneity test.

Discussion

In 1998, Motti and coworkers reported a positive relationship between homocysteine and

uric acid in an Italian population, particularly among individuals carrying *MTHFR* 677T allele [25]. Since then, the association of the *MTHFR* C677T polymorphism with hyperuricemia had been extensively investigated in several different ethnic populations, such as Japanese [11], Koreans [12], Chinese [14, 16] and Iranians [15]. All the studies conducted before 2008 showed that the *MTHFR* C677T polymorphism was significantly associated with an increased risk of hyperuricemia [11-18, 25]. A meta-analysis including 1,470 subjects by Wei and coworkers in 2012 also confirmed the significant association [10], although three studies by Wang *et al.* [16], Mai *et al.* [17] and Zhang *et al.* [18] were

not included in their meta-analysis. However, a recent study involving over 4,000 Japanese adults showed that the *MTHFR* C677T polymorphism was not associated with hyperuricemia

[19]. More recently, another large study with 902 Chinese participants also demonstrated that the association was nil [20]. The results of our current cross-sectional study were in line with the two large studies.

To systematically evaluate these controversial findings, we further combined our results and previously published findings using a Meta-analysis, which is a widely used statistical method in genetic epidemiological studies in an effort to achieve higher statistical power, to improve the precision of estimate and to settle uncertainty between conflicting studies. A total of 11 studies with 2,401 hyperuricemia cases and 7,101 individuals with normal uric acid levels were included in the final meta-analysis. The overall pooled estimates showed that the *MTHFR* C677T polymorphism was significantly associated with hyperuricemia risk, but the between-study heterogeneity was extremely high. The results of meta-regression analysis indicated that sample size and genotyping method might be the potential sources of heterogeneity. Further stratified analysis by sample size showed that the *MTHFR* C677T polymorphism was significantly associated with hyperuricemia in small study subgroup involving 1,438 subjects from seven studies, and the heterogeneity was very low ($I^2 = 2.7\%$). However, no significant association and high heterogeneity ($I^2 = 91.6\%$) were observed in large study subgroup, which included 8,046 participants from four studies. Large sample with enough participants is usually accompanied with lower selection bias and possess sufficient statistical power. This indicates that results from large studies are more precise and convincing. However, the extremely high heterogeneity presented in the large study subgroup in our meta-analysis substantially compromised the credibility of the evidences. When stratifying by genotyping method, the association was significant in polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) genotyping method studies but not in studies taking "others" as genotyping method. PCR-RFLP is the most commonly used genotyping method because of its simplicity. However, other genotyping methods including Taqman, gene chip, polymerase chain reaction with confronting two pair primers (PCR-CTPP) and polymerase chain reaction-ligase detection reaction (PCR-LDR), were usually reported to have

high accurate and sensitivity in single nucleotide polymorphism (SNP) genotyping [37-40]. In this meta-analysis, three studies in PCR-RFLP subgroup reported violated HWE, which usually indicates genotyping errors and thus questioned the accuracy of the pooled results in this subgroup. Additionally, we observed that three of four studies taking "others" as genotyping method have large sample size. Therefore, the significant association in small studies using PCR-RFLP method might be questionable.

Subsequent cumulative meta-analysis indicated that the three large recent studies have an apparent negative impact on positive association between the *MTHFR* C677T polymorphism and hyperuricemia. Although they did not change the direction of the association, they actually questioned the stability of previous accumulating evidence, which is also supported by the results of sensitivity analyses excluding studies with large sample size. The funnel plot and Egger's regression test showed that our study had significant publication bias, suggesting some studies without significant findings or with negative findings may be unpublished or missed. Moreover, according to recently proposed criteria for the assessment of cumulative evidence in genetic association studies, the significant association between the *MTHFR* C677T polymorphism and hyperuricemia can be characterized as having 'weak' epidemiological credibility. Therefore, further epidemiological studies with large sample size are still required to confirm or refute the significant relationship between the *MTHFR* C677T polymorphism and hyperuricemia susceptibility.

In interpreting the findings of the present study, two main limitations should be acknowledged. First, the participants in our cross-sectional study were from one hospital, which possess less representative of the general population. Second, due to the lack of detailed information on other genes and environmental factors such as diet and behavior habits, the gene-gene and gene-environmental interactions were not fully explored. Despite these limitations, our study still has several advantages. Firstly, our study has the largest sample sizes of all the studies that explored the association of the *MTHFR* C677T polymorphism with hyperuricemia in Chinese, which provides more representative

and authoritative reference data. Secondly, we adopted a comprehensive analysis strategy by combining cross-sectional study and meta-analysis together, which enlarges the sample size and strongly enhances the study power. Thirdly, we assigned a score for the epidemiological credibility of the findings. Fourthly, we performed secondary analyses including subgroup analysis, meta-regression test, cumulative meta-analysis and sensitivity analysis to explore the sources of heterogeneity and to assess the stability of the results.

In conclusion, the *MTHFR* C677T polymorphism was not associated with hyperuricemia risk in our cross-sectional study. Although a significant relationship was found in the following meta-analysis, it was found to have weak epidemiological credibility. Therefore, further well-designed studies with larger sample sizes are greatly needed to confirm the relationship between the *MTHFR* C677T polymorphism and hyperuricemia.

Acknowledgements

We gratefully acknowledge the assistance and cooperation of the faculty and staff of Dagang Oil Field Hospital and thank all of the participants in our study.

Disclosure of conflict of interest

None.

Address correspondence to: Guifan Sun, Research Center of Environment and Non-communicable Disease, School of Public Health, China Medical University, No. 77 Puhe Road, Shenyang North New Area, Shenyang 110122, P. R. China. Tel: +86-24-23261744; Fax: +86-24-23261744; E-mail: sungf@mail.cmu.edu.cn

References

[1] Richette P, Bardin T. Gout. *Lancet* 2010; 375: 318-328.

[2] Taniguchi A, Kamatani N. Control of renal uric acid excretion and gout. *Curr Opin Rheumatol* 2008; 20: 192-197.

[3] Koenig W, Meisinger C. Uric acid, type 2 diabetes, and cardiovascular diseases: fueling the common soil hypothesis? *Clin Chem* 2008; 54: 231-233.

[4] Li M, Hou W, Zhang X, Hu L, Tang Z. Hyperuricemia and risk of stroke: a systematic review

and meta-analysis of prospective studies. *Atherosclerosis* 2014; 232: 265-270.

[5] Qiu L, Cheng XQ, Wu J, Liu JT, Xu T, Ding HT, Liu YH, Ge ZM, Wang YJ, Han HJ, Liu J, Zhu GJ. Prevalence of hyperuricemia and its related risk factors in healthy adults from Northern and Northeastern Chinese provinces. *BMC Public Health* 2013; 13: 664.

[6] Yang B, Mo Z, Wu C, Yang H, Yang X, He Y, Gui L, Zhou L, Guo H, Zhang X, Yuan J, Dai X, Li J, Qiu G, Huang S, Deng Q, Feng Y, Guan L, Hu D, Zhang X, Wang T, Zhu J, Min X, Lang M, Li D, Hu FB, Lin D, Wu T, He M. A genome-wide association study identifies common variants influencing serum uric acid concentrations in a Chinese population. *BMC Med Genomics* 2014; 7: 10.

[7] Dehghan A, Kottgen A, Yang Q, Hwang SJ, Kao WL, Rivadeneira F, Boerwinkle E, Levy D, Hoffman A, Astor BC, Benjamin EJ, van Duijn CM, Witteman JC, Coresh J, Fox CS. Association of three genetic loci with uric acid concentration and risk of gout: a genome-wide association study. *Lancet* 2008; 372: 1953-1961.

[8] Kolz M, Johnson T, Sanna S, Teumer A, Vitart V, Perola M, Mangino M, Albrecht E, Wallace C, Farrall M, Johansson A, Nyholt DR, Aulchenko Y, Beckmann JS, Bergmann S, Bochud M, Brown M, Campbell H; EUROSPAN Consortium, Connell J, Dominiczak A, Homuth G, Lamina C, McCarthy MI; ENGAGE Consortium, Meitinger T, Mooser V, Munroe P, Nauck M, Peden J, Prokisch H, Salo P, Salomaa V, Samani NJ, Schlessinger D, Uda M, Völker U, Waeber G, Waterworth D, Wang-Sattler R, Wright AF, Adamski J, Whitfield JB, Gyllenstein U, Wilson JF, Rudan I, Pramstaller P, Watkins H; PROCARDIS Consortium, Doering A, Wichmann HE; KORA Study, Spector TD, Peltonen L, Völzke H, Nagaraja R, Vollenweider P, Caulfield M; WTCCC, Illig T, Gieger C. Meta-analysis of 28,141 individuals identifies common variants within five new loci that influence uric acid concentrations. *PLoS Genet* 2009; 5: e1000504.

[9] Köttgen A, Albrecht E, Teumer A, Vitart V, Krumsiek J, Hundertmark C, Pistis G, Ruggiero D, O'Seaghdha CM, Haller T, Yang Q, Tanaka T, Johnson AD, Kutalik Z, Smith AV, Shi J, Struchalin M, Middelberg RP, Brown MJ, Gaffo AL, Pirastu N, Li G, Hayward C, Zemunik T, Huffman J, Yengo L, Zhao JH, Demirkan A, Feitosa MF, Liu X, Malerba G, Lopez LM, van der Harst P, Li X, Kleber ME, Hicks AA, Nolte IM, Johansson A, Murgja F, Wild SH, Bakker SJ, Peden JF, Dehghan A, Steri M, Tenesa A, Lagou V, Salo P, Mangino M, Rose LM, Lehtimäki T, Woodward OM, Okada Y, Tin A, Müller C, Oldmeadow C, Putku M, Czamara D, Kraft P, Frogger L, Thun GA, Grotevendt A, Gislason GK, Harris TB, Launer LJ, McArdle P, Shuldiner

MTHFR C677T polymorphism and hyperuricemia

- AR, Boerwinkle E, Coresh J, Schmidt H, Schallert M, Martin NG, Montgomery GW, Kubo M, Nakamura Y, Tanaka T, Munroe PB, Samani NJ, Jacobs DR Jr, Liu K, D'Adamo P, Ulivi S, Rotter JI, Psaty BM, Vollenweider P, Waeber G, Campbell S, Devuyst O, Navarro P, Kolcic I, Hastie N, Balkau B, Froguel P, Esko T, Salumets A, Khaw KT, Langenberg C, Wareham NJ, Isaacs A, Kraja A, Zhang Q, Wild PS, Scott RJ, Holliday EG, Org E, Viigimaa M, Bandinelli S, Metter JE, Lupo A, Trabetti E, Sorice R, Döring A, Lattka E, Strauch K, Theis F, Waldenberger M, Wichmann HE, Davies G, Gow AJ, Bruinenberg M; LifeLines Cohort Study, Stolk RP, Kooner JS, Zhang W, Winkelmann BR, Boehm BO, Lucae S, Penninx BW, Smit JH, Curhan G, Mudgal P, Plenge RM, Portas L, Persico I, Kirin M, Wilson JF, Mateo Leach I, van Gilst WH, Goel A, Ongen H, Hofman A, Rivadeneira F, Uitterlinden AG, Imboden M, von Eckardstein A, Cucca F, Nagaraja R, Piras MG, Nauck M, Schurmann C, Budde K, Ernst F, Farrington SM, Theodoratou E, Prokopenko I, Stumvoll M, Jula A, Perola M, Salomaa V, Shin SY, Spector TD, Sala C, Ridker PM, Kähönen M, Viikari J, Hengstenberg C, Nelson CP; CARDIoGRAM Consortium; DIAGRAM Consortium; ICBP Consortium; MAGIC Consortium, Meschia JF, Nalls MA, Sharma P, Singleton AB, Kamatani N, Zeller T, Burnier M, Attia J, Laan M, Klopp N, Hillege HL, Kloiber S, Choi H, Pirastu M, Tore S, Probst-Hensch NM, Völzke H, Gudnason V, Parsa A, Schmidt R, Whitfield JB, Fornage M, Gasparini P, Siscovick DS, Polašek O, Campbell H, Rudan I, Bouatia-Naji N, Metspalu A, Loos RJ, van Duijn CM, Borecki IB, Ferrucci L, Gambaro G, Deary IJ, Woffenbuttel BH, Chambers JC, März W, Pramstaller PP, Snieder H, Gyllenstein U, Wright AF, Navis G, Watkins H, Witteman JC, Sanna S, Schipf S, Dunlop MG, Tönjes A, Ripatti S, Soranzo N, Toniolo D, Chasman DI, Raitakari O, Kao WH, Ciullo M, Fox CS, Caulfield M, Bochud M, Gieger C. Genome-wide association analyses identify 18 new loci associated with serum urate concentrations. *Nat Genet* 2013; 45: 145-154.
- [10] Wei W, Liu SY, Zeng FF, Ma L, Li KS, Wang BY. Meta-analysis of the association of the C677T polymorphism of the methylenetetrahydrofolate reductase gene with hyperuricemia. *Ann Nutr Metab* 2012; 60: 44-51.
- [11] Zuo M, Nishio H, Lee MJ, Maejima K, Mimura S, Sumino K. The C677T mutation in the methylenetetrahydrofolate reductase gene increases serum uric acid in elderly men. *J Hum Genet* 2000; 45: 257-262.
- [12] Hong YS, Lee MJ, Kim KH, Lee SH, Lee YH, Kim BG, Jeong B, Yoon HR, Nishio H, Kim JY. The C677T mutation in methylenetetrahydrofolate reductase gene: correlation with uric acid and cardiovascular risk factors in elderly Korean men. *J Korean Med Sci* 2004; 19: 209-213.
- [13] Ding LL. Study on the risk factors of hyperuricemia among males and related gene polymorphisms analysis. Master thesis, Xinjiang Medical University 2005.
- [14] Shi HY, Dong YH, Nan HR, Qian WW, Qian RL. Association of the methylenetetrahydrofolate reductase (*MTHFR*) gene C677T polymorphism and hyperuricemia. *Chin J Diabetes* 2006; 14: 178-81.
- [15] Golbahar J, Aminzadeh MA, Al-Shboul QM, Kassab S, Rezaian GR. Association of methylenetetrahydrofolate reductase (C677T) polymorphism with hyperuricemia. *Nutr Metab Cardiovasc Dis* 2007; 17: 462-467.
- [16] Wang F, Zhao SH, Yan SL, Wang YG, Li CG. Association of methylenetetrahydrofolate reductase gene C677T polymorphism with hyperuricemia. *Chin J Endocrinol Metab* 2007; 23: 62-63.
- [17] Mai Y, Shou T, Tang H, Wu RD, Xu CY, Ding W, Li Y, Yin XM. Relationship between *MTHFR* C677T polymorphism and hyperuricemia. *Chinese Journal of Medical Guide* 2008; 10: 365-366.
- [18] Zhang QH, Liu J. Methylenetetrahydrofolate reductase C677T polymorphism for primary hyperuricemia and gouty arthritis. *Chin J Gen Pract* 2008; 7: 259-260.
- [19] Hinohara Y, Naito M, Okada R, Yin G, Higashibata T, Tamura T, Kawai S, Morita E, Wakai K, Matsuo H, Mori A, Hamajima N. No association between *MTHFR* C677T and serum uric acid levels among Japanese with ABCG2 126QQ and SLC22A12 258WW. *Nagoya J Med Sci* 2013; 75: 93-100.
- [20] Pan YY. Association of the methylenetetrahydrofolate reductase polymorphism with hyperuricemia. Master thesis, Ningxia Medical University 2014.
- [21] Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M, Kluijtmans LA, van den Heuvel LP, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet* 1995; 10: 111-113.
- [22] Lussier-Cacan S, Xhignesse M, Piolot A, Selhub J, Davignon J, Genest J Jr. Plasma total homocysteine in healthy subjects: sex-specific relation with biological traits. *Am J Clin Nutr* 1996; 64: 587-593.
- [23] Malinow MR, Levenson J, Giral P, Nieto FJ, Razavian M, Segond P, Simon A. Role of blood pressure, uric acid, and hemorheological parameters on plasma homocyst(e)ine concentration. *Atherosclerosis* 1995; 114: 175-183.
- [24] Cohen E, Levi A, Vecht-Lifshitz SE, Goldberg E, Garty M, Krause I. Assessment of a possible

MTHFR C677T polymorphism and hyperuricemia

- link between hyperhomocysteinemia and hyperuricemia. *J Investig Med* 2015; 63: 534-538.
- [25] Motti C, Gnasso A, Bernardini S, Massoud R, Pastore A, Rampa P, Federici G, Cortese C. Common mutation in methylenetetrahydrofolate reductase. Correlation with homocysteine and other risk factors for vascular disease. *Atherosclerosis* 1998; 139: 377-383.
- [26] Yang B, Fan S, Zhi X, Wang Y, Wang Y, Zheng Q, Sun G. Prevalence of hyperhomocysteinemia in China: a systematic review and meta-analysis. *Nutrients* 2014; 7: 74-90.
- [27] Yang B, Liu Y, Li Y, Fan S, Zhi X, Lu X, Wang D, Zheng Q, Wang Y, Wang Y, Sun G. Geographical distribution of *MTHFR* C677T, A1298C and *MTRR* A66G gene polymorphisms in China: findings from 15357 adults of Han nationality. *PLoS One* 2013; 8: e57917.
- [28] Fu S, Luo L, Ye P, Xiao W. Epidemiological associations between hyperuricemia and cardio-metabolic risk factors: a comprehensive study from Chinese community. *BMC Cardiovasc Disord* 2015; 15: 129.
- [29] Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med* 2002; 21: 1539-1558.
- [30] Zintzaras E, Ioannidis JP. Heterogeneity testing in meta-analysis of genome searches. *Genet Epidemiol* 2005; 28: 123-137.
- [31] DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986; 7: 177-188.
- [32] Thompson SG, Sharp SJ. Explaining heterogeneity in meta-analysis: a comparison of methods. *Stat Med* 1999; 18: 2693-2708.
- [33] Trikalinos TA, Salanti G, Zintzaras E, Ioannidis JP. Meta-analysis methods. *Adv Genet* 2008; 60: 311-334.
- [34] Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997; 315: 629-634.
- [35] Ioannidis JP, Boffetta P, Little J, O'Brien TR, Uitterlinden AG, Vineis P, Balding DJ, Chokalingam A, Dolan SM, Flanders WD, Higgins JP, McCarthy MI, McDermott DH, Page GP, Rebbeck TR, Seminara D, Khoury MJ. Assessment of cumulative evidence on genetic associations: interim guidelines. *Int J Epidemiol* 2008; 37: 120-132.
- [36] Thakkinstian A, McElduff P, D'Este C, Duffy D, Attia J. A method for meta-analysis of molecular association studies. *Stat Med* 2005; 24: 1291-1306.
- [37] Tharinjaroen CS, Intorasoot S, Anukool U, Phunpae P, Butr-Indr B, Orrapin S, Sangboonruang S, Arunothong S, Chaiyasirinroj B, Kunyanone N, Kasinrerak W, Tragoolpua K. Novel Targeting, *lepB* Gene, Using Polymerase Chain Reaction With Confronting Two Pair Primers (PCR-CTPP) for Simultaneous Detection of *Mycobacterium tuberculosis* complex and *Mycobacterium bovis*. *J Med Microbiol* 2016; 65: 36-43.
- [38] Pingle MR, Granger K, Feinberg P, Shatsky R, Sterling B, Rundell M, Spitzer E, Larone D, Golightly L, Barany F. Multiplexed identification of blood-borne bacterial pathogens by use of a novel 16S rRNA gene PCR-ligase detection reaction-capillary electrophoresis assay. *J Clin Microbiol* 2007; 45: 1927-1935.
- [39] Heller T, Kirchheiner J, Armstrong VW, Luthe H, Tzvetkov M, Brockmüller J, Oellerich M. AmpliChip CYP450 GeneChip: a new gene chip that allows rapid and accurate CYP2D6 genotyping. *Ther Drug Monit* 2006; 28: 673-677.
- [40] Zhou C, Ni J, Zhao Y, Su B. Rapid detection of epidermal growth factor receptor mutations in non-small cell lung cancer using real-time polymerase chain reaction with TaqMan-MGB probes. *Cancer J* 2006; 12: 33-39.

MTHFR C677T polymorphism and hyperuricemia

Table S1. Association of the *MTHFR* C677T polymorphism with hyperuricemia among males

Polymorphism	Hyperuricemia (n = 338)	Normal (n = 1139)	Crude OR (95% CI)	P	Adjusted OR ^a (95% CI)	P
Codominant						
CC	76 (22.49)	244 (21.42)	1.00	-	1.00	-
CT	174 (51.48)	537 (47.15)	1.04 (0.76-1.42)	0.802	0.90 (0.65-1.26)	0.548
TT	88 (26.04)	358 (31.43)	0.79 (0.56-1.12)	0.182	0.72 (0.49-1.04)	0.078
Dominant						
CC	76 (22.49)	244 (21.42)	1.00	-	1.00	-
CT + TT	262 (77.51)	895 (78.58)	0.94 (0.70-1.26)	0.677	0.83 (0.60-1.13)	0.237
Recessive						
CC+CT	250 (73.96)	781 (68.57)	1.00	-	1.00	-
TT	88 (26.04)	358 (31.43)	0.77 (0.58-1.01)	0.058	0.77 (0.57-1.03)	0.079
Allelic						
C	326 (48.22)	1025 (45.00)	1.00	-	-	-
T	350 (51.78)	1253 (55.00)	0.92 (0.75-1.12)	0.377	-	-

OR, odds ratio; CI, confidence index. ^aAdjusted by age, creatinine, triglycerides, total cholesterol, diastolic blood pressure and body mass index.

Table S2. Association of the *MTHFR* C677T polymorphism with hyperuricemia among females

Polymorphism	Hyperuricemia (n = 46)	Normal (n = 696)	Crude OR (95% CI)	P	Adjusted OR ^a (95% CI)	P
Codominant						
CC	8 (17.39)	158 (22.70)	1.00	-	1.00	-
CT	27 (58.70)	321 (46.12)	1.66 (0.74-3.74)	0.220	1.20 (0.51-2.80)	0.677
TT	11 (23.91)	217 (31.18)	1.00 (0.39-2.55)	0.998	0.55 (0.20-1.55)	0.258
Dominant						
CC	8 (17.39)	158 (22.70)	1.00	-	1.00	-
CT + TT	38 (82.61)	538 (77.30)	1.40 (0.64-3.05)	0.405	0.94 (0.41-2.15)	0.891
Recessive						
CC + CT	35 (76.09)	479 (68.82)	1.00	-	1.00	-
TT	11 (23.91)	217 (31.18)	0.69 (0.35-1.39)	0.303	0.48 (0.22-1.06)	0.070
Allelic						
C	43 (46.74)	637 (45.76)	1.00	-	-	-
T	49 (53.26)	755 (54.24)	0.97 (0.60-1.56)	0.889	-	-

OR, odds ratio; CI, confidence index. ^aAdjusted by age, creatinine, triglycerides and body mass index.

MTHFR C677T polymorphism and hyperuricemia

Table S3. Baseline characteristics of the studies included in the meta-analysis

Author (year) [Ref.]	Country (Ethnicity)	Study design	Genotyping Method	Age, years	Source of Controls	Diagnosis Criteria	Gender
Zuo <i>et al.</i> (2000) [11]	Japan (East Asian)	Cross sectional	PCR-RFLP	52.6 ± 8.8	PB	≥ 417 mg/dl	Males
Hong <i>et al.</i> (2004) [12]	Korean (East Asian)	Cross sectional	PCR-RFLP	51.87 (40-81)	PB	≥ 417 mg/dl	Males
Lili Ding (2005) [13]	China (East Asian)	Case-control	Gene-chip	Case: 38.89 ± 8.45 Control: 37.77 ± 8.30	HB	> 420 μmol/L	Males
Shi <i>et al.</i> (2006) [14]	China (East Asian)	Case-control	PCR-RFLP	Case: 55 ± 11 Control: 54 ± 12	PB	Males: > 420 μmol/L Females: > 360 μmol/L	Males/females
Golbahar <i>et al.</i> (2007) [15]	Iran (Iranian)	Cross sectional	PCR-RFLP	Females: 44.5 ± 15.8 Males: 50.1 ± 16.2	PB	Females: > 285.6 mmol/L Males: > 315.4 mmol/L	Males/females
Wang <i>et al.</i> (2007) [16]	China (East Asian)	Case-control	PCR-RFLP	Case: 43.9 ± 11.2 Control: 43.4 ± 12.3	HB	Males: > 417 μmol/L Females: > 356 μmol/L	Males/females
Mai <i>et al.</i> (2008) [17]	China (East Asian)	Case-control	PCR-RFLP	Case: 60.6 ± 16.0 Control: 60.7 ± 12.0	HB	≥ 416 μmol/L	Males
Zhang <i>et al.</i> (2008) [18]	China (East Asian)	Case-control	PCR-RFLP	-	HB	Males: > 417 μmol/L Females: > 356 μmol/L	Males/females
Hinohara <i>et al.</i> (2013) [19]	Japan (East Asian)	Cross sectional	PCR-CTPP	Females: 49.2 ± 8.7 Males: 50.7 ± 8.6	PB	≥ 417 mg/dl	Females
Yuyang Pan (2014) [20]	China (East Asian)	Case-control	PCR-LDR	Case: 49.33 ± 10.24 Control: 50.79 ± 10.20	HB	Males: > 417 μmol/L Females: > 357 μmol/L	Males/females
Present study	China (East Asian)	Cross sectional	TaqMan	Females: 45.64 ± 9.34 Males: 47.43 ± 10.04	HB	Males: ≥ 360 μmol/L Females: ≥ 420 μmol/L	Males/females

PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; PCR-CTPP, polymerase chain reaction with confronting two pair primers; PCR-LDR, polymerase chain reaction-ligase detection reaction; HB, hospital based; PB, population based.

MTHFR C677T polymorphism and hyperuricemia

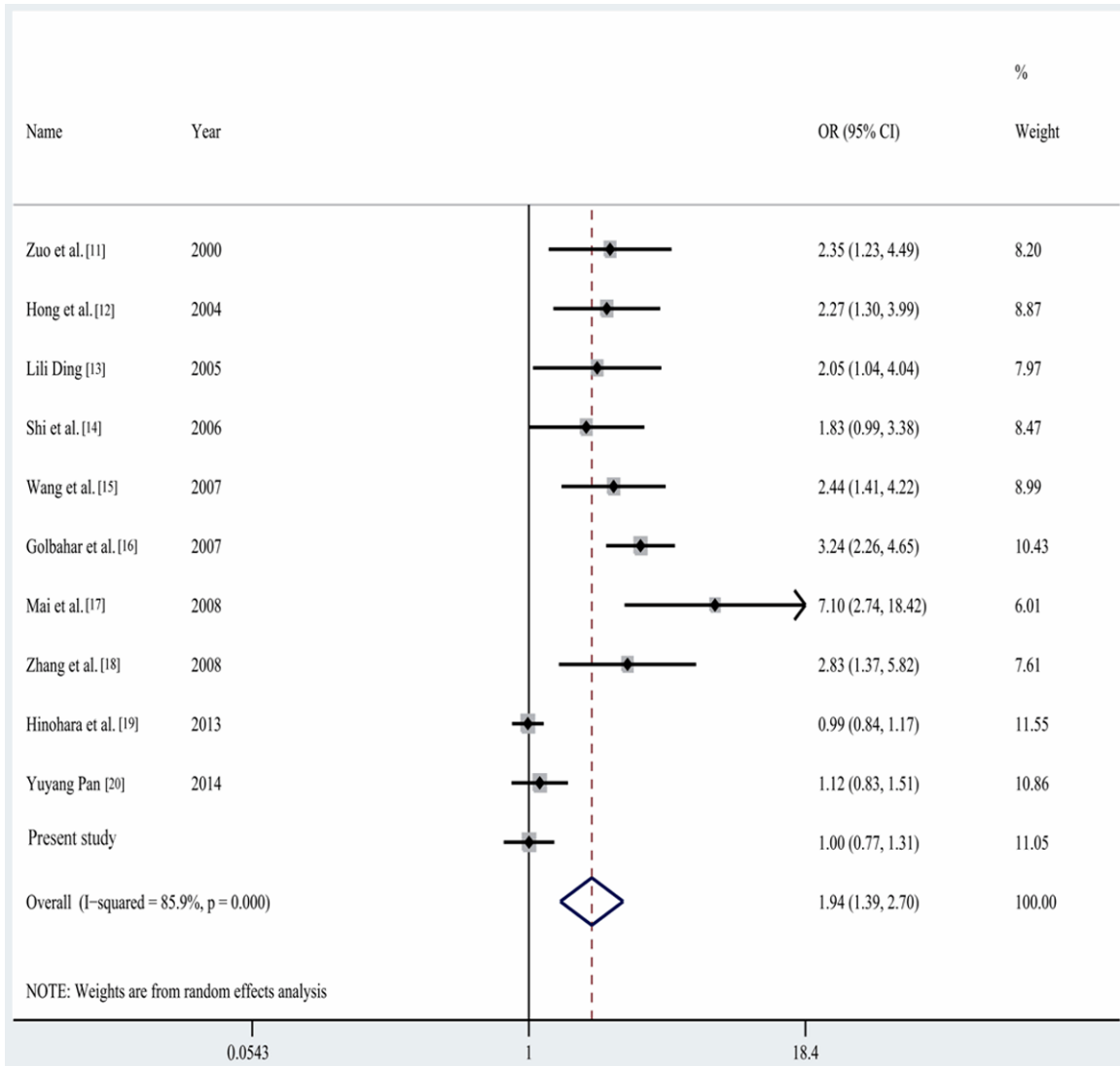


Figure S1. Forest plot of the association between *MTHFR* C677T polymorphism and hyperuricemia in the dominant model (TT + CT vs. CC).

MTHFR C677T polymorphism and hyperuricemia

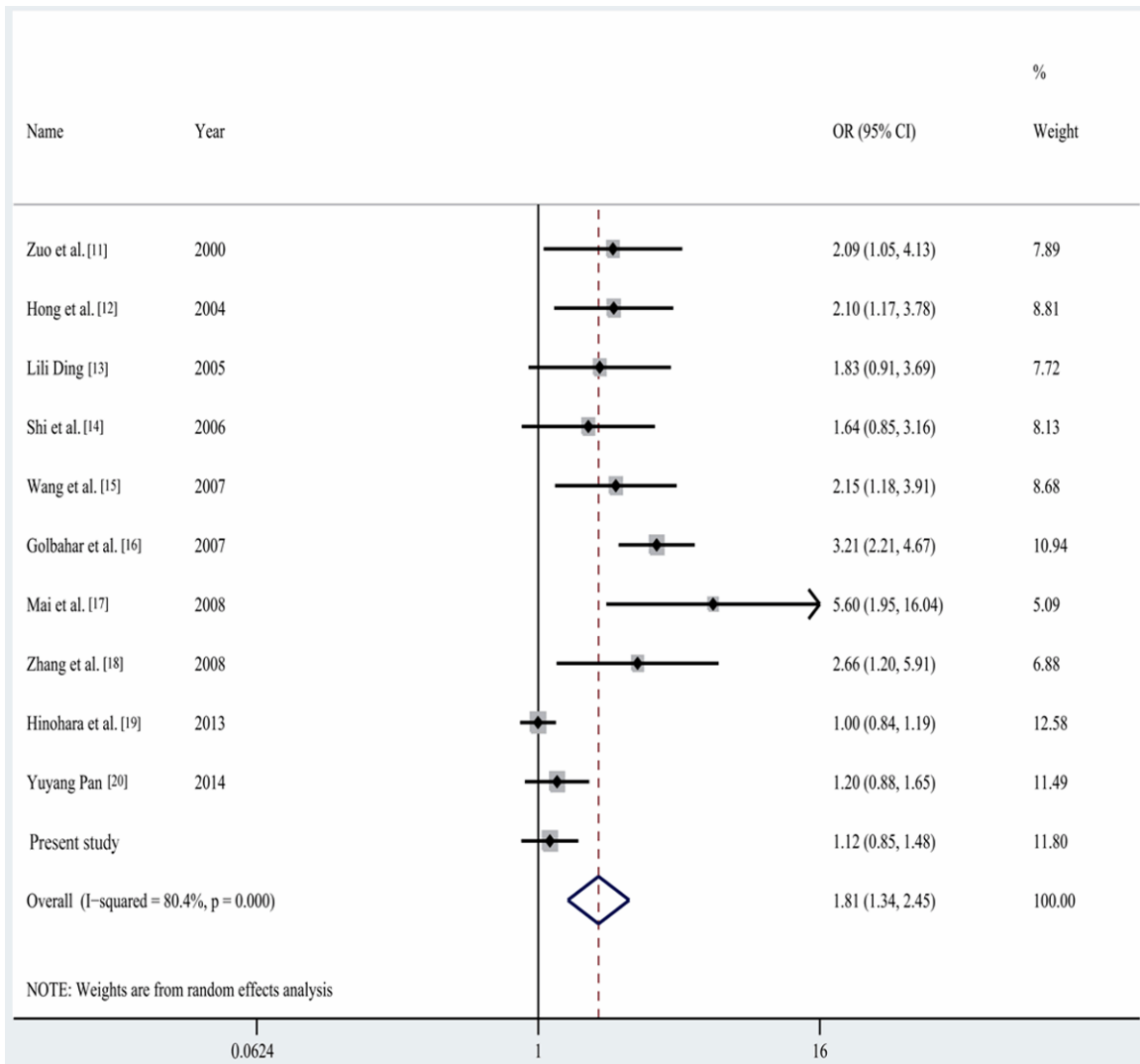


Figure S2. Forest plot of the association between *MTHFR* C677T polymorphism and hyperuricemia in the heterozygous codominant model (CT vs. CC).

MTHFR C677T polymorphism and hyperuricemia

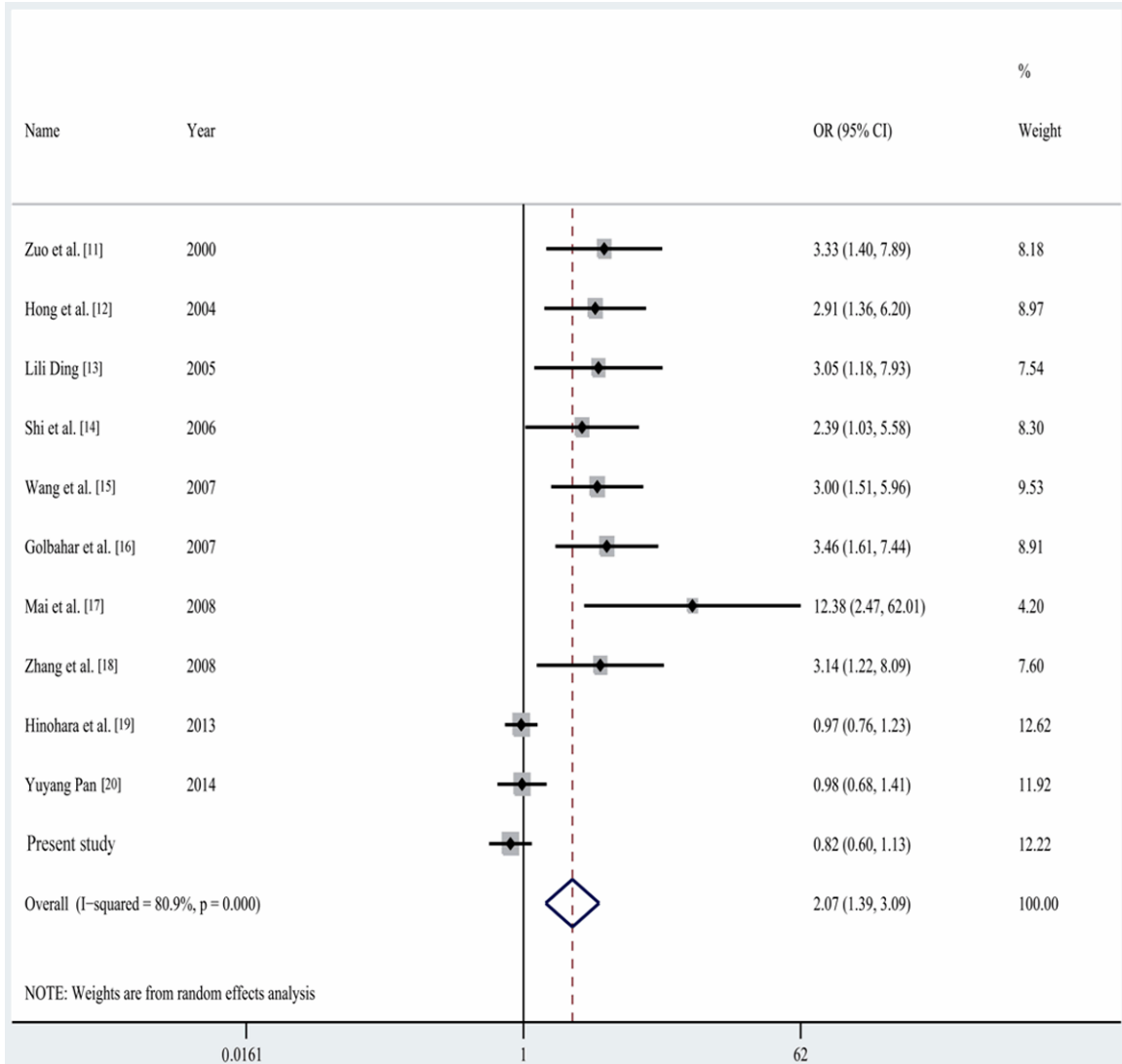


Figure S3. Forest plot of the association between *MTHFR* C677T polymorphism and hyperuricemia in the homozygous codominant model (TT vs. CC).

MTHFR C677T polymorphism and hyperuricemia

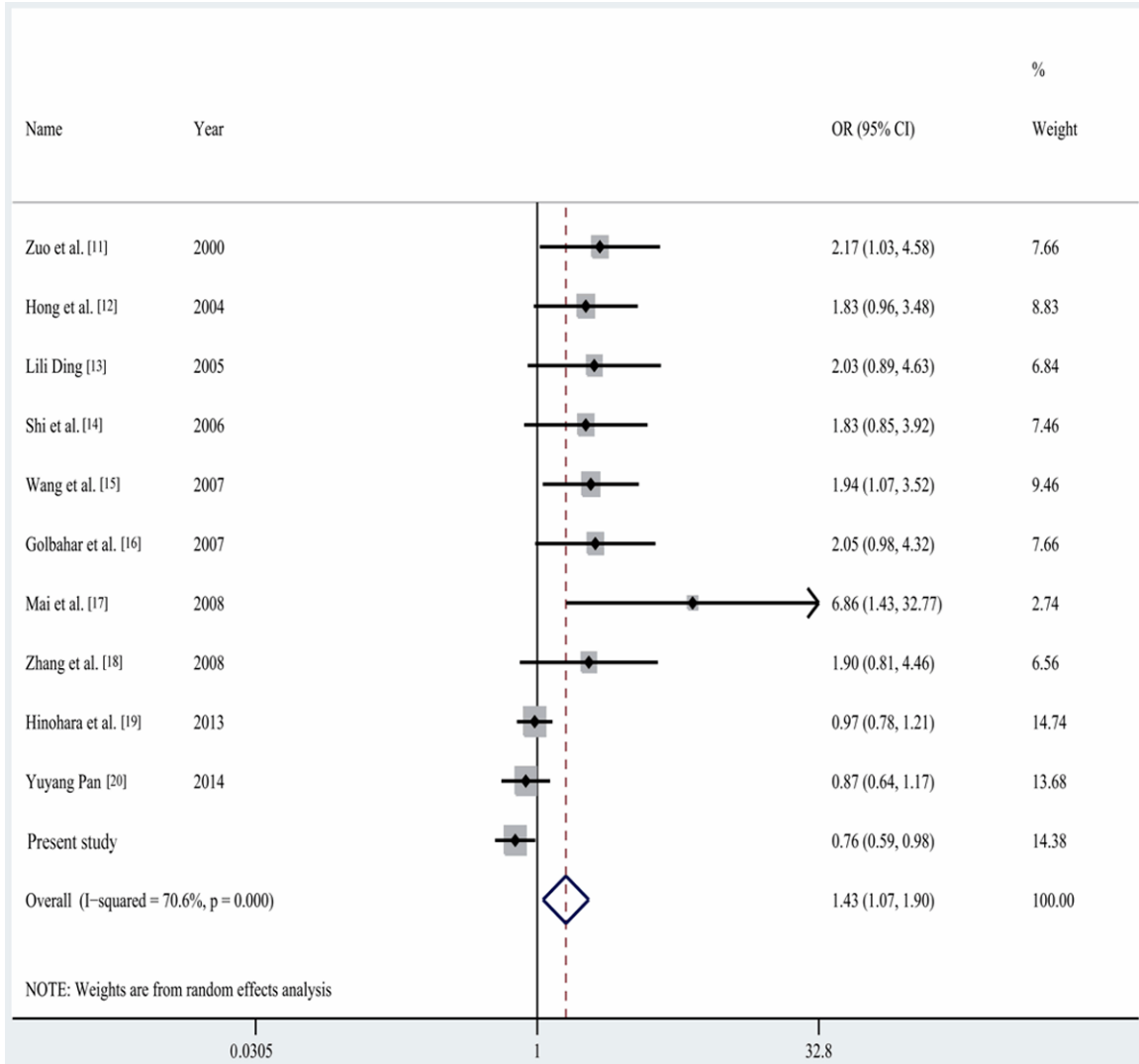


Figure S4. Forest plot of the association between *MTHFR* C677T polymorphism and hyperuricemia in the recessive model (TT vs. CT + CC).

MTHFR C677T polymorphism and hyperuricemia

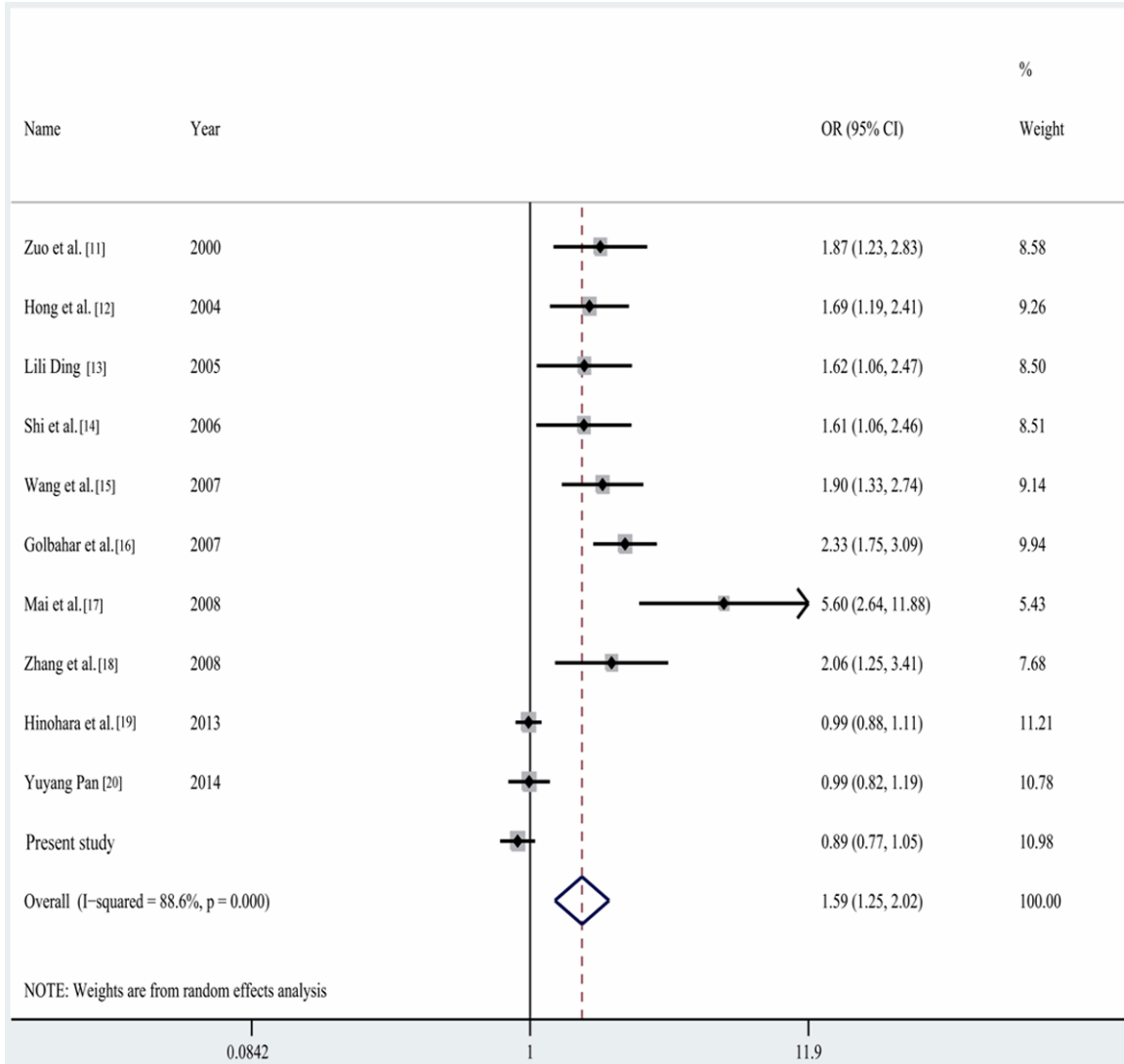


Figure S5. Forest plot of the association between *MTHFR* C677T polymorphism and hyperuricemia in the allelic model (T vs. C).

MTHFR C677T polymorphism and hyperuricemia

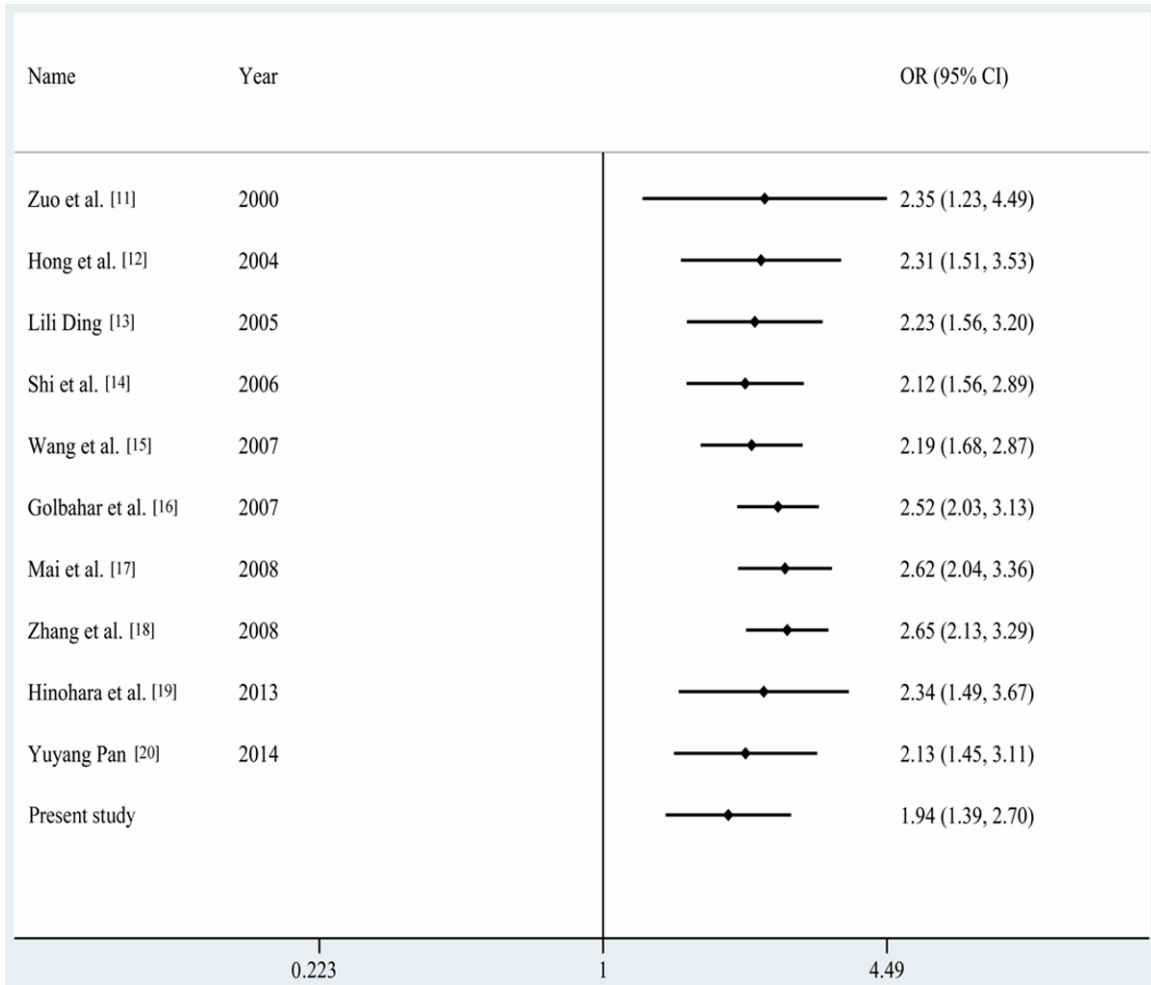


Figure S6. The cumulative forest plot of OR with 95% CI for the *MTHFR* C677T polymorphism and hyperuricemia in the dominant model.

MTHFR C677T polymorphism and hyperuricemia

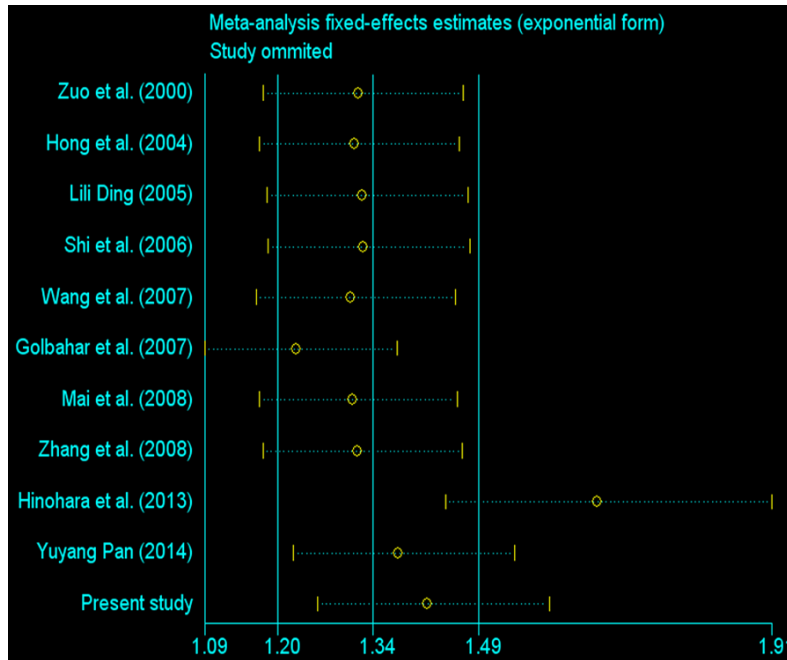


Figure S7. Sensitivity analysis of the association of *MTHFR* C677T polymorphism with hyperuricemia in the dominant model.

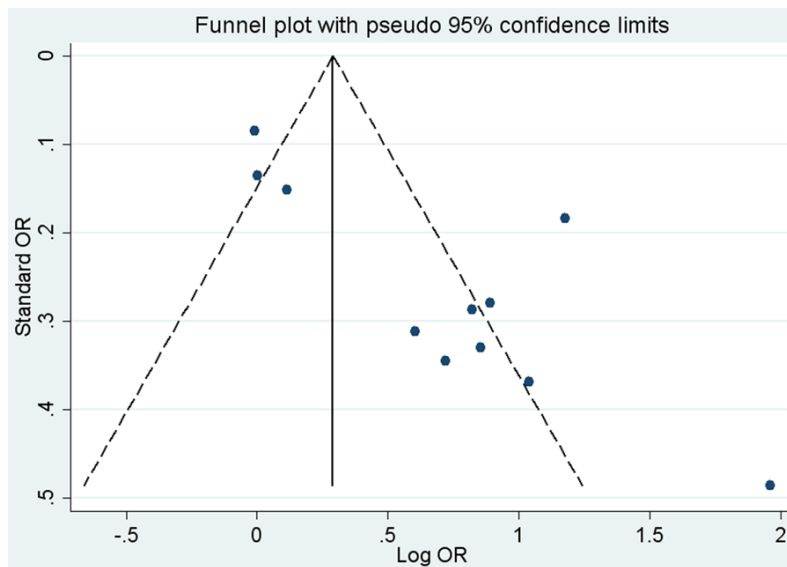


Figure S8. Funnel plot analysis on the detection of publication bias in the meta-analysis of the association between the *MTHFR* C677T polymorphism and hyperuricemia in the dominant model.