

# The MTHFR C677T Polymorphism and Hyperuricemia Risk: a Meta-analysis of 558 Cases and 912 Controls

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## Abstract

Both genetic and environmental factors play roles in hyperuricemia and susceptibility may be modified by functional polymorphisms in folate metabolic genes, such as methylenetetrahydrofolate reductase (MTHFR). Several case control studies investigated association between C677T polymorphism with hyperuricemia but the sample size was small in these studies and the association power was weak. The aim of the present meta-analysis was to evaluate association between MTHFR C677T polymorphism and hyperuricemia. This meta-analysis recruited 6 published studies which were selected by search of electronic databases up to August 2013, including 558 hyperuricemic cases and 912 healthy controls. Odds ratios (ORs) with 95% confidence intervals (CIs) were used to assess the association between MTHFR C677T polymorphism and hyperuricemia susceptibility using fixed effect models. Statistically significant relationship was found between C677T polymorphism and hyperuricemia with all genetic models (Additive model T vs. C: OR=1.8401, 95% CI=1.55-2.18,  $p<0.0001$ ; Homozygote model TT vs. CC: OR=2.9873, 95% CI=2.06-4.33,  $p<0.0001$ ; Co-dominant CT vs. CC: OR=2.3785, 95% CI=1.85-3.04,  $p<0.0001$ ; Dominant model TT+CT vs. CC: OR=2.5233, 95% CI=1.99-3.19,  $p<0.0001$ ; Recessive model TT vs. CT+CC: OR=2.2628, 95% CI=1.61-3.17,  $p<0.0001$ ). In conclusion, the MTHFR C677T polymorphism was associated with an increased risk of hyperuricemia.

**Keyword:** Hyperuricemia; MTHFR; C677T; Homocysteine; Meta-analysis

**Abbreviation:** MTHFR: Methylenetetrahydrofolate Reductase

## Introduction

Many clinical and epidemiological studies have reported that higher serum uric acid (UA) is related to increased risk of gout, obesity, hypertension hyperlipidemia, renal insufficiency, insulin resistance, cardiovascular and cerebrovascular diseases in general population [1-3]. Elevated serum UA was commonly detected in subjects with abnormal purine metabolism, reflecting overproduction of UA and/or insufficient UA excretion from the kidney. On average, serum UA increases with age [1,4] and is higher in men than in women possibly because of estrogens [5,6] and is considered as a marker of renal dysfunction, as well as a risk factor of renal disease progression.

The uric acid is the end product of purines catabolism and hyperuricemia is the result of imbalance among production (liver) and excretion (renal and fecal) of 70% and 30%, respectively. In appropriate concentrations, uric acid acts as an antioxidant and in high concentrations (hyperuricemia), acts as pro-oxidant [7,8]. Smoking, alcohol intake, purine-rich foods and the renal hypoexcretion resulting from drugs like diuretics are the major causes of hyperuricemia. In addition, several genetic factors have been associated with hyperuricemia. Methylenetetrahydrofolate reductase (MTHFR) gene polymorphism (C677T) is reported as one of these genetic factors in several studied [9-11].

MTHFR is an essential enzyme in metabolizing folate, which catalyzes 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. The product is the dominant form of circulating folate, and provides a methyl group for the remethylation of homocysteine back to methionine. MTHFR gene is composed of twelve exons and is localized on chromosome 1p36.3 [10]. C677T Transition (rs1801133) is a common mutation in the coding region of the MTHFR gene that causes an alanine to valine (Al222Val) substitution [10]. This mutation is associated with reduced MTHFR activity and higher homocysteine levels. Homozygosity for the mutation (TT) predisposes to significantly

elevated plasma homocysteine levels [10]. The frequency of the MTHFR 677T allele varies in different ethnic and regional world populations for example, the allele frequency is 0.07 in Sub-Saharan Africans and 0.06 in Canadian Inuit, whereas in Asians the allele frequencies are 0.04-0.54 (11,23,26). Although the mechanism of the relationship between MTHFR polymorphism and serum uric acid is still unknown, some studies have presumed that the MTHFR mutation could affect mechanisms such as the de novo synthesis of purines via 10-formyl tetrahydrofolate with consequent overproduction of UA by the substrate of the MTHFR reaction.

In present meta-analysis, the estimates of the genetic association of each individual study and a pooled estimate were obtained. In addition, the heterogeneity between studies and the existence of publication bias were investigated.

## Methods

Author assessed the association between the MTHFR C677T polymorphism and hyperuricemia by conducting meta-analysis of all published papers and pooled analysis of individual-level data available.

## Search strategy and identification of relevant studies

Author searched Pubmed, Google Scholar and Springer Link databases online to identify potential relevant epidemiological publications through August 2013 and used the key term

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“Methylenetetrahydrofolate Reductase”, “MTHFR”, “C677T” as well as “hyperuricemia” in the search.

Those fulfilling the following criteria were considered eligible for further analysis: 1. Examining the exact topic of concern; 2. Published work with access; 3. Case-control study providing the individual numbers of all three genotypes of MTHFR (CC, CT and TT) from both case and control groups, or providing gene frequencies and sample sizes, or sufficient data for measuring OR and corresponding 95%CI.

### Data extraction

Following information were extracted from each study: 1, name of first author; 2, year of publication; 3, journal name; 4, study design based on the background of control individuals; 5, country; 6, total sample size and distribution of each genotype in case and control group respectively.

### Statistical analysis

Crude ORs with 95% CIs were used to assess the strength of association between the MTHFR C677T polymorphism and hyperuricemia risk. The pooled ORs were performed with additive/allele contrast model (T vs. C), co-dominant model (CT vs. CC), homozygote model (TT vs. CC), dominant model (CT+TT vs. CC), and recessive model (TT vs. CC+CT), respectively. Both fixed-effects model [12-14] and random effects [15] model were applied in measurements.

Author also assessed heterogeneity between studies by chi-square-based Q-test and I<sup>2</sup> statistics [16], and a P value less than 0.05 for the Q-test or I<sup>2</sup> value greater than 50% in I<sup>2</sup> statistics indicates the existence of heterogeneity between studies, then random-effects model was better for interpretation, otherwise, chose the fixed-effects model results. Additionally, Chi-squared test was used to determine if the observed frequencies of genotypes conformed to Hardy-Weinberg equilibrium expectations.

### Publication bias

Publication bias was investigated with the funnel plot. Funnel plot asymmetry was further assessed by the method of Egger’s linear regression test [17]. Analyses were performed using the computer

program MIX version 1.7 [ 18]. A p value less than 0.05 was considered statistically significant, and all the p values were two sided.

## Results

### Characteristics of included studies

According to our search strategy and inclusion criteria, five studies with full-text articles were remained the relationship of MTHFR C677T polymorphism with hyperuricemia [19-23]. One author [21] reported male and female samples separately, so author treated them independently. Hence total six studies were found suitable for the inclusion in present meta-analysis. The studies were published between 2000 and 2007. All these five studies were performed in different countries- Korea [20], China [ 22,23] , Iran [21], and Japan [19]. All samples belong to two ethnic populations Caucasian and Asian (Table 1). In all six studies, total cases were 558 with CC (168), CT (299) and TT (91), and controls were 912 with CC (451), CT (371), and TT (90) genotypes. In controls genotypes, percentage of CC, CT and TT were 49.45 %, 40.68%, and 9.87% respectively. In total cases, genotype percentage of CC, CT, and TT was 30.11%, 53.58% and 16.31% respectively. Frequencies of CC and CT genotypes were highest in both cases and controls (Table 2). In cases and controls, the allele C was the most common. Control samples in all studies were in Hardy Weinberg equilibrium.

### Frequency of risk allele in the control population

To estimate the pooled frequency, author combined case-control studies of MTHFR C677T and extracted data only from the control group. Based on all these samples, the frequency of risk T allele varied among different ethnicities i.e. in Caucasian and Asian: high in Asian healthy populations 35.36% and low in Caucasian healthy populations 19%.

### Meta-analysis using allele frequency

Mutant T allele showed significant association with hyperuricemia in both fixed effect (OR=1.8401, 95% CI=1.55-2.18, p<0.0001) and random effect (OR=1.4401, 95% CI=1.55-2.18, p<0.0001) models (Table 3 and Figure 1).

Study	Country	Control	Case	Year	Reference
Zuo et al.	Japan	213	58	2000	J Hum Genet, 45: 257-262.
Hong et al.	Korea	240	87	2004	J Korean Med Sci, 19: 209-213.
Gobahar et al (male)	Iran	153	115	2006	Nutr Metab Cardiovasc Dis, 17: 462-467
Golbahar et al.(Female)	Iran	134	116	2006	Nutr Metab Cardiovasc Dis, 17: 462-467
Shi et al.	China	91	90	2006	Chin J Diabetes, 14: 178-181.
Yao et al.	China	81	92	2007	Ai Bian Ji Bian Tu Bian, 19: 50-52.

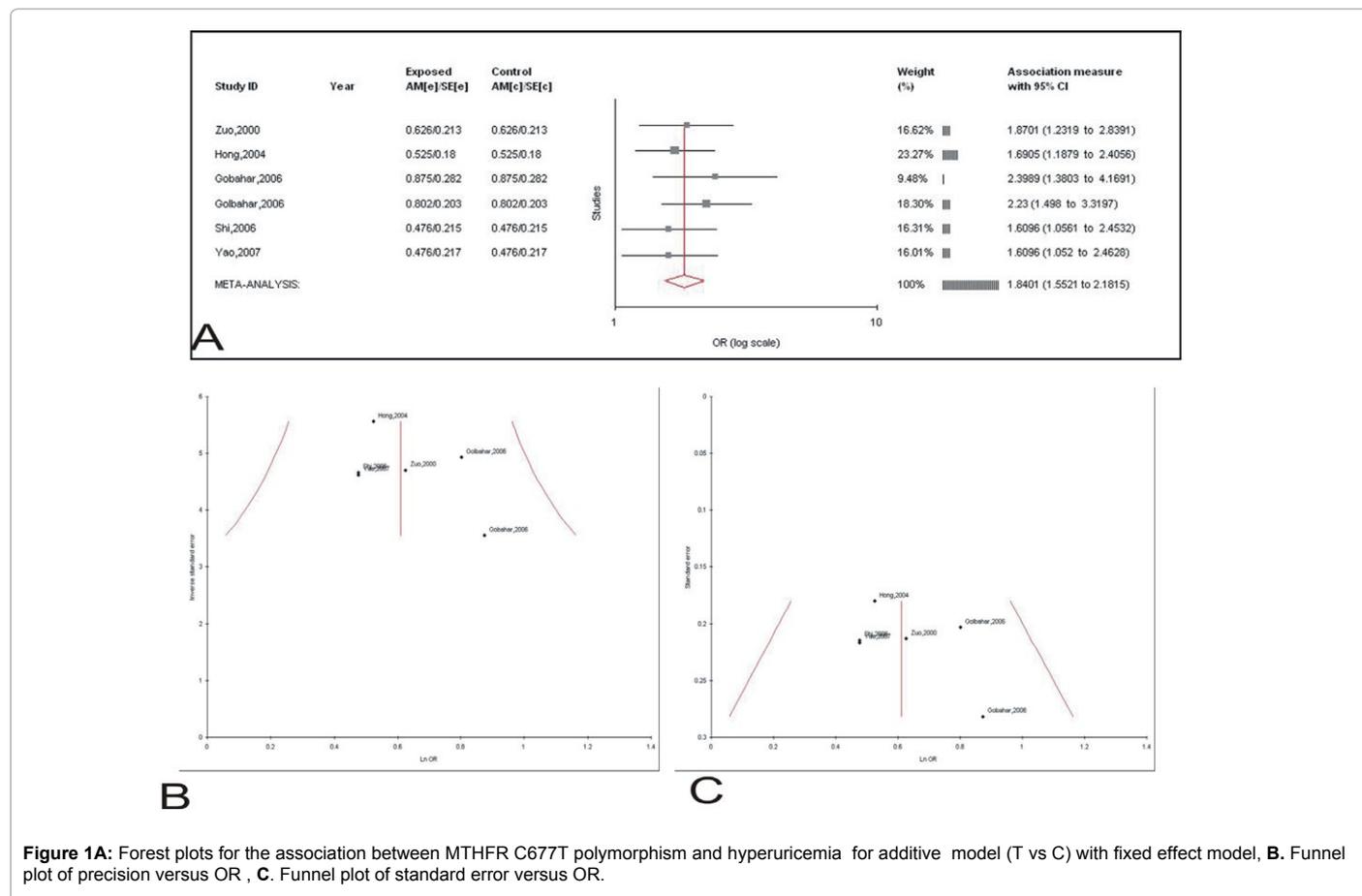
Table 1: Characteristics of six studies included in the present meta-analysis.

Study ID	Genotype						Alleles			
	CC		CT		TT		C		T	
	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
Zuo, 2000	15	96	30	92	13	25	60	284	56	142
Hong, 2004	20	97	49	113	18	30	89	307	85	173
Gobahar, 2006 (male)	48	106	57	41	10	6	153	253	77	53
Golbahar, 2006 (Female)	39	84	68	44	9	6	146	212	86	56
Shi, 2006	27	40	42	38	21	13	96	118	84	64
Yao, 2007	19	28	53	43	20	10	91	99	93	63

Table 2: The distributions of MTHFR C677T genotypes and allele frequencies in hyperuricemic patients and healthy controls.

Genetic Models	Fixed effect OR (95% CI), p	Random effect OR (95% CI), p	Heterogeneity p-value (Q test)	I <sup>2</sup> (%)	Publication Bias (p of Egger's test)
Allele Contrast (T vs C)	1.84 (1.55-2.18), <0.0001	1.44 (1.55-2.18), <0.0001	0.734	0%	0.323
Dominant (TT+CT vs CC)	2.52 (1.99-3.19), <0.0001	2.53 (1.99-3.20), <0.0001	0.634	0%	0.050
Homozygote (TT vs CC)	2.98 (2.06-4.33), <0.0001	3.00 (2.06-4.24), <0.001	0.992	0%	0.283
Co-dominant (CT vs CC)	2.38 (1.85-3.04), <0.0001	2.39 (1.86-3.05), <0.0001	0.477	0%	0.022
Recessive (TT vs CT+CC)	2.26 (1.61-3.17), <0.0001	2.27 (1.61-3.17), <0.00001	0.996	0%	0.886

**Table 3:** Summary estimates for the odds ratio (OR) of MTHFR C677T in various allele/genotype contrasts, the significance level (p value) of heterogeneity test (Q test), I<sup>2</sup> metric and publication bias p-value (Egger Test).



### Meta-analysis using genotype frequency

Similar to allele meta-analysis, pooled odds ratio for mutant genotypes (CT+TT) showed statistically significant association with hyperuricemia adopting both fixed (OR=2.5233, 95% CI=1.99-3.19, p<0.0001) and random (OR=2.5303, 95% CI=1.99-3.20, p<0.0001) effect models (Figure 2). Association was also detected between the C677T polymorphism and the susceptibility to hyperuricemia in all other three genetic models (for TT vs. CC (homozygote model): OR=2.9873, 95% CI=2.06-4.33, p<0.0001; for TT vs. CT + CC (recessive model): OR=2.2628, 95% CI=1.61-3.17, p<0.0001; for CT vs. CC (codominant model): OR=2.3785, 95% CI=1.85-3.04, p<0.0001) (Table 3 and Figure 3).

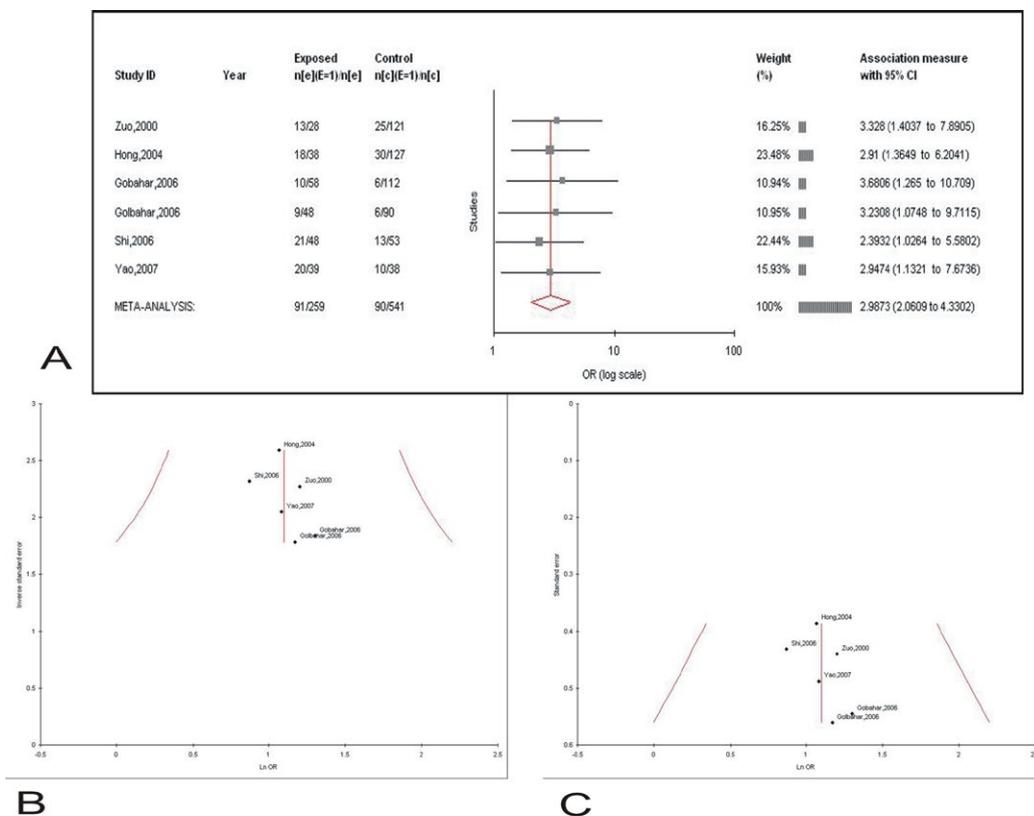
### Sensitivity analysis

In the sensitivity analysis, the influence of each study on the pooled OR was examined by repeating the meta-analysis while omitting each

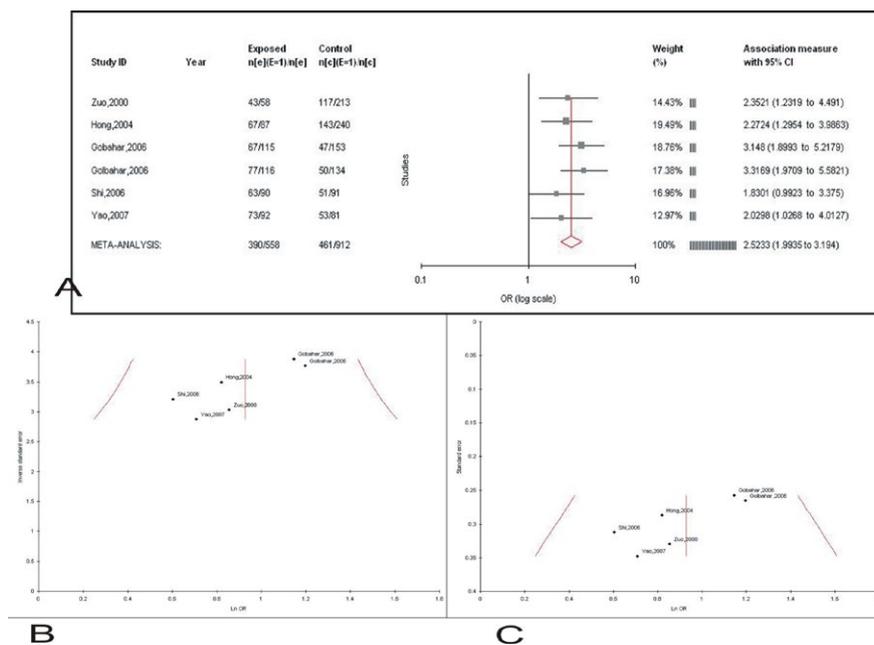
study, one at a time. This procedure confirmed the stability of present meta-analysis result (data not shown).

### Publication bias

Funnel plots were generated using standard error and precision values and log OR using fixed effect models (Figures 1-3). Symmetrical distribution of studies in the funnel plots suggests absence of publication bias. This is also supported by other tests. Begg's funnel plot and the Egger's test were conducted to estimate the publication bias of articles. Both the results of Begg's and Egger's test did not show any evidence of publication bias except co-dominant model (T vs C Begg's test, P=1.000, Egger's test, P=0.323; CT vs. CC Begg's test, P=0.259, Egger's test, P=0.022; TT vs CC Begg's test, P=0.259, Egger's test, P=0.283; Dominant model, Begg's test, P=0.259, Egger's test, P=0.05; Recessive model, Begg's test, P=1.000, Egger's test, P=0.886) (Table 3 and Figure 3).



**Figure 2 A:** Forest plots for the association between MTHFR C677T polymorphism and hyperuricemia for homozygote model (TT vs CC) with fixed effect model, **B.** Funnel plot of precision versus OR, **C.** Funnel plot of standard error versus OR.



**Figure 3 A:** Forest plots for the association between MTHFR C677T polymorphism and hyperuricemia for dominant model (TT+CT vs CC) with fixed effect model, **B.** Funnel plot of precision versus OR, **C.** Funnel plot of standard error versus OR.

## 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 and during past decade several meta-analyses were published assessing MTHFR as risk factor to various diseases/disorders like Cardiovascular disease [24], diabetes [25], Cancer [11] and psychiatric disorder [26-28] etc. The present meta-analysis examining MTHFR C677T in 558 patient and 912 control subjects indicated that carriers of the T allele and TT genotype are at a statistically significant increased risk of hyperuricemia.

Motti and coworkers reported significant association between MTHFR C677T polymorphism and serum UA in Italian population [29]. Their study showed that in TT individuals there was a positive correlation between homocysteine and serum uric acid concentrations. In 2000, Zuo et al. reported C677T polymorphism as risk factor for hyperuricemia in older Japanese subjects. Similar results were observed by Hong et al. in Korean population. Golbahar et al. carried out this analysis in male and female samples separately and found significant association between UA and MTHFR C677T polymorphism in both groups. On the other hand, there were 2 studies [22,23] reporting association between the MTHFR 677TT genotype and SUA mean in Chinese.

MTHFR 677T allele is associated with higher homocysteine concentration [30-36]. Dysfunction of homocysteine metabolism has been linked to several disorders, including neural tube defects (NTDs) [37], stroke [38] and syndromes [39]. Significant association between hyperhomocysteinemia and serum uric acid was reported in atherosclerotic patients in several studies [38].

Higher concentration of homocysteine was risk factor for vascular diseases/atherosclerosis, and in TT subjects due to higher homocysteine concentration, vascular disease or renovascular atherosclerosis occurs and clearance of uric acid may reduce resulting in elevation of serum uric acid [39]. In addition adenosine originating from S-adenosyl-homocysteine, and preferentially incorporated into a precursor pool for uric acid, and would link the syntheses of homocysteine and uric acid [12,29].

The limitations of the present meta-analysis are (i) small number of studies (only six studies), (ii) small sample size (1470 samples), (iii) different ethnic backgrounds of the individuals included in the study, (iv) widely spread exclusion and inclusion criteria which might complicate the comparison between the studies. Undoubtedly, all the limitations will inevitably affect the summary results, but the strength of present meta-analysis is based on the accumulating studies, and thus has much better power to reach a more precise estimation.

In conclusion, results of present meta-analysis suggest that the MTHFR C677T allele is significant risk of hyperuricemia. Future large-scale, population-based association studies are now required to investigate potential gene-gene and gene-environment interactions involving the MTHFR C677T polymorphism in determining hyperuricemia risk. The sample size was rather small and further studies with large number of subjects are necessary for confirming the result of this meta-analysis.

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