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ORIGINAL ARTICLE

Methylenetetrahydrofolate reductase gene polymorphism and risk of chronic myelogenous leukemia: a meta-analysis

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Summary

Purpose: *Reported evidence supports a role for methylenetetrahydrofolate reductase (MTHFR) in the risk of chronic myelogenous leykemia (CML). However, these reports arrived at non-conclusive and even conflicting results regarding the association between two common MTHFR polymorphisms (C677T and A1298C) and CML risk. Thus, a meta-analysis was carried out to clarify a more precise association between these two polymorphisms and the CML risk by updating the available publications.*

Methods: *Pooled odds ratios (OR) with corresponding 95% confidence interval (95% CI) and stratification analysis were performed to estimate the relationship between MTHFR polymorphisms and the risk of CML under different genetic comparison models.*

Results: *Data from the meta-analysis showed no significant association between MTHFR C677T polymorphism and CML risk. However, significant associations were found between MTHFR A1298C variants and CML risk under homozygous comparison model (CC vs AA, OR=1.62, 95% CI=1.11–2.36, p=0.01) and dominant comparison model (CC+AC vs AA, OR=1.68, 95% CI=1.17–2.43, p=0.005) in overall population; especially more obvious impacts were noticed for Asian populations in subgroup analysis for homozygous model (CC vs AA, OR=2.00, 95% CI=1.25–3.21, p=0.004) and dominant model (CC+AC vs AA, OR=2.49, 95% CI=1.42–4.36, p=0.001), but this did not apply in Caucasian populations.*

Conclusion: *The results of this meta-analysis suggested no significant association between MTHFR C677T polymorphism and CML risk, while an increased CML risk was noticed for 1298C variant carriers, especially in Asian populations but not in Caucasian populations, which suggested ethnicity differences between MTHFR A1298C polymorphisms and risk of CML.*

Key words: *CML, gene polymorphism, MTHFR, tumor marker*

Introduction

CML is a myeloproliferative malignancy characterized by accumulation of myeloid precursors in bone marrow, peripheral blood and body tissues [1]. Studies have shown that 95% of patients with CML possess the Philadelphia chromosome which results from a reciprocal translocation t (9;22) (q31;q11) between chromosome 9 and chromosome 22. The translocation results in the formation of a fusion gene that encodes Bcr-Abl protein, which possesses aberrant protein tyrosine kinase activity. This, in turn, activates its downstream signaling pathways involving cell proliferation, metastasis, cell survival, and differentiation. Progression of CML patients is divided

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into three phases, namely chronic phase, accelerated phase and blast crisis phase. CML patients with no treatment will develop fatal blast crisis phase within 5 years [2-5].

Although the biological and clinical aspects of CML have been well documented, little is known about the mechanism underling the genesis of Philadelphia chromosome and the factors influencing individuals' susceptibility to CML. To date, there have been no reports on familial, geographic, hereditary, ethnic and economic association with the pathogenesis of CML, and the mechanisms behind the disease progression haven't been fully understood as well [6]. It is recognized that leukemia is derived from abnormally proliferating hematopoietic tissue with greatest DNA synthesis, and thought the pathogenesis of CML would be associated with the metabolic fate of folic acid [7]. Approximately 30 enzymes are involved in folate metabolism, among them, MTHFR being a pivotal enzyme. It catalyzes the irreversible reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is the predominant circulatory form of folate and the carbon donor for remethylation of homocysteine to methionine at expanse of DNA synthesis [8]. Folate deficiency can result in DNA hypomethylation and misincorporation of uracil during DNA replication, which can lead to DNA double-strand breaks during excision repair process [9,10]. Two common MTHFR single nucleotide polymorphic sites had been identified, namely C677T (rs1801133) and A1298C (rs1801131). C677T is a transition of cytosine to thymine at position 677 which results in a substitution of alanine to valine at codon 222 of the enzyme, while the A1298C mutation causes a glutamate to alanine substitution of the enzyme at codon 429. Studies have shown reduced enzyme activity of both MTHFR polymorphisms (C677T and A1298C). MTHFR 677CT and 677TT genotypes constitute the 60% and 30% enzyme activity of the wide type genotype 677CC, respectively. A1298C variants also result in reduced enzyme activity. Reduced activity of the MTHFR enzyme leads to high concentration of homocysteine in plasma, and breaks the folate metabolic balance which increases the availability of 5,10-methylenetetrahydrofolate for thymine synthesis, thus reducing the uracil misincorporation during DNA synthesis [11-13]. In this view, MTHFR polymorphisms (C677T and A1298C) would probably modulate the efficiency of methylation process and DNA synthesis, and thus would explain the individuals' susceptibility to different kinds of cancer and hematological malignancies [14,15].

The association between MTHFR polymorphisms (C677T and A1298C) and the susceptibility to CML was investigated in the past decade. However, these studies [16-23] arrived at inconclusive and even conflicting results, partly because each study had small sample size and insufficient statistical power to demonstrate a significant association. Moreover, each individual study only investigated a single population, making results difficult to interpret [16-28]. Hence, we carried out a meta-analysis to possibly reach a significant and conclusive relationship between the MTHFR C677T/A1298C polymorphisms and the risk of development of CML. To determine this association, pooled data from update case-control studies regarding the MTHFR C677T/A1298C polymorphisms with adult CML were analyzed.

Methods

Publication search and selection criteria

Embase, PubMed, Web of Science, Cochrane Library and CNKI (China National Knowledge Infrastructure) were searched using the combining terms: ("Methylenetetrahydrofolate reductase" or "MTHFR" or "C677T" or "A1298C") and ("Chronic Myelogenous Leukemia" or "CML") and ("polymorphism" or "folate" or "mutation" or "leukemia") for studies addressing the relationship between MTHFR C677T and/or A1298C polymorphisms and risk of adult CML. The publication search applied an upper date limit of July 8, 2014. Only studies with full text were included, and the search was carried out with no restriction on language but only focusing on studies that had been conducted on human subjects. We also searched the reference lists of retrieved articles and reviews for relevant studies. Only the complete and the most recent studies were chosen if there were more than one instance of the same patient population included in several studies. The following criteria were applied for inclusion of the eligible studies: (1) case-control designed studies; (2) studies investigating the relationship between MTHFR C677T and/or A1298C polymorphism and CML; (3) sufficient genotype frequencies of MTHFR polymorphism were available for estimating the OR. The reasons for studies' exclusion were: (1) review articles; (2) case-only studies; (3) studies with overlapping data. Twenty-two assessment items of the Strengthening the reporting of genetic association studies (STREGA) system were applied for the quality appraisal of each study, giving scores ranging from 0 to 22. The studies were classified into three levels depending on their scores: high quality (18-22), moderate qual-

ity (13-17), and low quality (0-12). As a whole, this meta-analysis was carried out according to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA).

Data extraction

The following information including first author, publication year, country of origin, ethnicity of study population, numbers of the controls and cases for C677T and/or A1298C genotypes were carefully extracted from each eligible study independently by two authors according to the inclusion criteria listed above. We didn't set a minimum number of patients as a criterion for a study's inclusion in the meta-analysis. Disagreements were resolved by reaching a consensus among all authors.

Statistics

For the control group of each study, the genotype distribution was assessed for Hardy-Weinberg Equilibrium (HWE) using x^2 test, and $P_{HWE} < 0.05$ showed statistical significance. If the study was found not to be in HEW it was excluded from the meta-analysis. Overall, the OR with 95% CI were calculated to examine the strength of the association between MTHFR polymorphisms and CML risk. Pooded ORs were estimated using both the Fixed effects and Random effects models. With the ORs, forest plot was generated by the software Review Manager 5.0. Four different genetic comparison models, namely allele comparison model ("T vs C" or

Table 1. Study characteristics

First author	*Publication year*	*Ethnicity (Country)*	*Polymorphisms studied*	*Case*		*Control*		*STREGA score*
				Number	*Mean age (years)*	*Number*	*Mean age (years)*	
Deligezer [18]	2003	Caucasian (Turkey)	C677T	132(F/M = 70/61)	45.9	161(F/M=112/48)	39	17
Hur [19]	2006	Asian (Korea)	C677T, A1298C	40(F/M = 13/27)	50	200(F/M=72/128)	34	17
Moon [26]	2007	Asian (Korea)	C677T, A1298C	115(F/M = 40/75)	43.8	434(F/M=238/195)	41.4	18
Barbosa [17]	2008	Caucasian (Brazil)	C677T, A1298C	67(F/M = 30/36)	44	100(F/M=47/53)	29	18
Kim [24]	2009	Asian (Korea)	C677T, A1298C	149(F/M = 55/94)	50.4	1700(F/M=879/821)	52.2	19
Ismail [27]	2009	Asian (Jordan)	C677T, A1298C	149(NA)	NA	170(NA)	NA	16
Vahid [28]	2010	Asian (Iran)	C677T, A1298C	38(F/M = 19/19)	45	97(F/M=50/47)	44.8	17
Jankovic [22]	2011	Caucasian (Serbia)	C677T	43(NA)	NA	26(NA)	NA	15
Hussain [20]	2012	Asian (India)	C677T	43(NA)	39.5	251(NA)	41.5	16
Lordelo [25]	2012	Caucasian (Brazil)	C677T, A1298C	41(NA)	NA	155(NA)	NA	15
Lordelo [21]	2012	Mixed (Brazil)	C677T, A1298C	64(NA)	NA	118(NA)	NA	14
Jakovljevic [23]	2012	Caucasian (Serbia)	C677T	52(F/M = 24/28)	NA	53(F/M=24/30)	NA	18
Khorshied [16]	2014	Caucasian (Egypt)	C677T, A1298C	97(F/M = 51/46)	NA	130(F/M=68/62) NA		19
Rabab [16]	2014	Caucasian (Egypt)	C677T, A1298C	85(F/M = 40/45)	46.7	100(F/M=49/51) 48.2		18

NA: not available, F: female, M: male. STREGA (Strengthening the reporting of genetic association studies) system includes 22 items, giving scores ranging from 0 to 22

Table 2. Distribution of methylenetetrahydrofolate reductase (MTHFR) C667T and A1298C genotypes

First author		*Frequency of C677T MTHFR genotype*							*Frequency of A1298C MTHFR genotype*			
		CC		*CT*		*TT*		P_{HWE}	*AA*	*AC*	*CC*	P_{HWE}
Deligezer [18]	Case	72	74	51	73	9	14	0.501				
	Control											
Hur [19]	Case	13	80	17	80	10	40	0.018	31	7	2	
	Control								116	78	6	0.094
Moon [26]	Case	43	144	45	196	27	94	0.078	74	33	8	
	Control								307	120	7	0.219
Barbosa [17]	Case	46	65	19	29	2	6	0.27	41	23	3	
	Control								63	32	5	0.722
Kim [24]	Case	54	540	72	863	26	297	0.133	97	49	5	
	Control								1147	500	53	0.868
Ismail [27]	Case	63	94	67	66	19	10	0.722	59	68	22	
	Control								76	81	13	0.172
Vahid [28]	Case	24	56	11	37	3	4	0.487	12	19	7	
	Control								39	36	22	0.021
Jankovic [22]	Case	17	6	21	16	5	4	0.225				
	Control											
Hussain [20]	Case	28	180	8	61	7	10	0.106				
	Control											
Lordelo [25]	Case	15	74	21	66	5	15	0.959	26	15	0	
	Control								68	79	8	0.013
Lordelo [25]	Case	31	66	26	48	7	4	0.179	35	28	1	
	Control								51	64	3	0.001
Jakovljevic [21]	Case	18	13	29	33	5	7	0.057				
	Control											
Khorshied [23]	Case	41	65	45	52	11	13	0.587	54	37	6	
	Control								55	65	10	0.121
Rabab [16]	Case	30	45	44	49	11	6	0.119	32	38	15	
	Control								40	51	9	0.199

P_{HWE}: P value for Hardy-Weinberg equilibrium in the control group; one study for MTHFR C677T polymorphism and three studies for MTHFR A1298C polymorphism were excluded from meta-analysis because $P_{HWE} < 0.05$.

"C vs A"), comparisons of homozygous model ("TT vs CC" or "CC vs AA"), dominant model ("TT + TC vs CC" or "CC + AC vs AA") and recessive model ("CC + CT vs TT" or "AA +AC vs CC") were employed for estimation of the association between MTHFR C677T and/or A1298C polymorphisms and CML risk, respectively. Subgroup analysis also was performed among Caucasians, Asians and mixed ethnicities. Heterogeneity between studies was estimated using x^2-based Q statistic, and p value <0.10 was considered statistically significant. To determine the proportion of variability resulting from between-study heterogeneity, I^2 metric statistic was calculated, and 25% < I^2, 25% < I^2 <50% and 75% < I^2 were interpreted as low, moderate and large or extreme heterogeneity, respectively. Possible publication bias was estimated by Begg's funnel plot, in which the standard error of log (Logor) of each study was plotted against log. Asymmetry of the funnel plot suggested a possible publication bias. Furthermore, Begg's funnel plot asymmetry was tested by the method of Egger's linear regression, which is a linear regression approach to measure funnel plot asymmetry on the natural logarithmic scale of odds ratio (OR). Significance of intercept was estimated using the t-test suggested by Egger's test, and p<0.05 was considered as a potential statistical publication bias. Sensitivity analyses were carried out to examine whether the individual study influenced the pooled results. Most calculations in this meta-analysis were performed using the Review Manager 5.0 software. The sensitivity and Egger's test were estimated using the software Stata, version 12.0. (Stata Corp, College Station, TX, USA).

Results

Study characteristics

In accordance with the study inclusion criteria listed above, we found 14 eligible case-control studies addressing the relationship between MTHFR C677T/A1298C polymorphism and CML. Figure 1 illustrates the process for study search and inclusion, and the reasons for exclusion. The

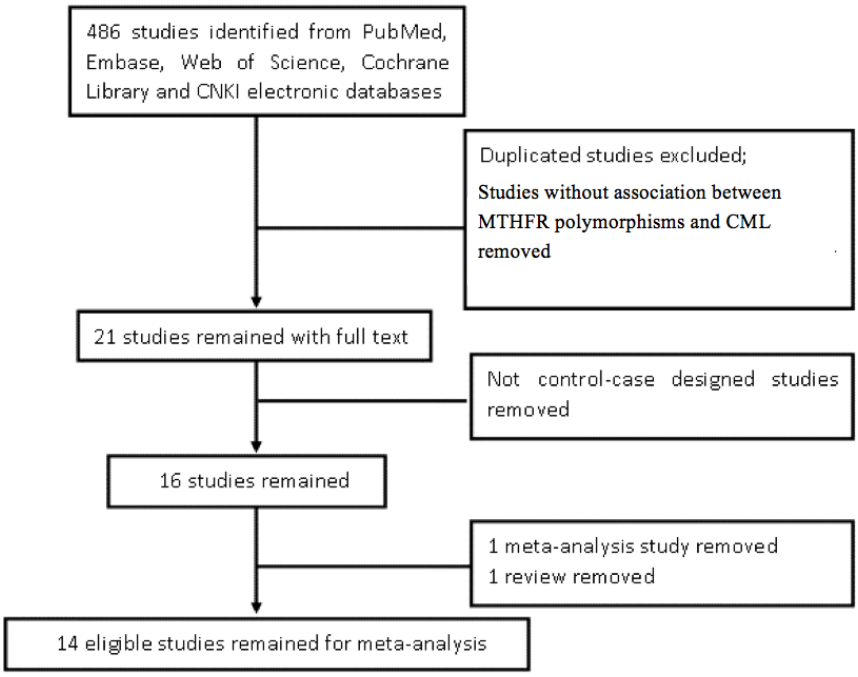

Figure 1. Flow diagram for study inclusion in the meta-analysis and reasons for study exclusion.

publication year of these studies ranged from 2003 to 2014. Study quality assessment with STREGA system showed these studies were of moderate to high quality, with STREGA scores more than 13 (Table 1). In all studies, the patients were molecularly and histologically confirmed, and control groups were free of CML and matched for gender and age. The studies were conducted in various populations of different ethnicities (Table 1), with 7 on Caucasian population [16-18, 21-23, 25], 6 on Asian population [19,20,24,26-28] and 1 on mixed population [25] for MTHFR C677T polymorphism, and with 5 on Asian population [19,24,26-28], 4 on Caucasian population [16,17,23,25] and 1 on mixed population [25] for MTHFR A1298C polymorphism. 1118 cases and 3695 controls were found in these studies for MTHFR C677T polymorphism. The allele and variants genotype frequency of each study are listed in Table 2. In the case group, 44% homozygous CC-individuals, 43% CT-heterozygous and 13% TT-homozygous individuals demonstrated MTHFR C677T polymorphism. For the control group of C677T polymorphism, homozygous CC-individuals, CT-heterozygous and TT-homozygous individuals were 41, 45 and 14%, respectively. The 677T allele frequencies in case and control groups were 34 and 37%, respectively.

In terms of A1298C polymorphism, a total of 847 cases and 3204 controls were found in the studies. For the case group, 54% AA-homozygous individual, 37% AC-heterozygous and 8% CC-homozygous individuals illustrated the MTHFR A1298C polymorphism, while in the control group, 61, 35 and 4% were for AA-homozygous individuals, AC-heterozygous individuals and CC-homozygous individuals, respectively. 1298C allele frequencies for the case group and the control group were 27 and 22%, respectively. The genotype distribution in the control group of all studies was examined by the x^2 test for HWE, and the results showed one study for MTHFR C677T polymorphism [19] and two studies [25,28] for MTHFR A1298C polymorphism weren't consistent with HWE, because $P_{HWE}<0.05$ (Table 2), indicating genotyping error and/or ethnicity stratification. These three studies were excluded from the subsequent meta-analysis to clarify the effect of MTHFR C677T/A1298C on the risk of CML.

Meta-analysis results

Overall, Table 3 lists the main results of this meta-analysis. For the MTHFR C677T polymorphism, we didn't find significant associations between CML risk and the C677T polymorphism under the allele model (Figure 2A). Furthermore, comparisons using homozygous (TT vs CC), dominant (TT vs [CT+CC]) and recessive (TT+CT vs CC) models produced the same pattern of results as

Table 3. Meta analysis for association between MTHFR C677T/A1298C polymorphisms and CML risk under different comparison models

	Ethnicity	*OR* *Fixed effects (95% CI)*	*P*	*Random effects (95% CI)*	*P*	I^2 %	*P value Q text*
C677T (rs1801133)							
Alleles (T vs C)	All	1.07 (0.96 – 1.20)	0.24	1.09 (0.92 – 1.29)	0.31	49	0.02
	Caucasian	1.00 (0.84 – 1.19)	1.00	0.99 (0.78 – 1.26)	0.95	41	0.12
	Asian	1.09 (0.94 – 1.27)	0.27	1.16 (0.88 – 1.53)	0.29	64	0.02
	Mixed	1.23 (0.83 – 1.83)	0.31	1.23 (0.83 – 1.83)	0.31	–	–
TT vs CC	All	1.21 (0.95 – 1.54)	0.13	1.33(0.91 – 1.96)	0.15	50	0.02
	Caucasian	1.05 (0.69 – 1.60)	0.56	1.03(0.63 – 1.69)	0.89	19	0.28
	Asian	1.24 (0.91 – 1.68)	0.17	1.61 (0.87 – 2.99)	0.13	68	0.01
	Mixed	3.37 (1.01 – 13.68)	0.05	3.37 (1.01 – 13.68)	0.05	–	–
TT vs (CT + CC)	All	1.24(0.99 – 1.55)	0.06	1.34 (0.98 – 1.83)	0.07	35	0.10
	Caucasian	1.03 (0.69 – 1.53)	0.90	1.03 (0.69 – 1.55)	0.89	0	0.61
	Asian	1.29 (0.98 – 1.71)	0.07	1.62 (0.96 – 2.74)	0.07	62	0.03
	Mixed	3.50 (0.98 – 12.45)	0.05	3.50 (0.98 – 12.45)	0.05	–	–
(TT + CT) vs CC	All	1.03 (0.88 – 1.20)	0.72	1.04 (0.85 – 1.27)	0.72	37	0.08
	Caucasian	0.99 (0.78 – 1.25)	0.94	0.98 (0.71 – 1.36)	0.92	42	0.11
	Asian	1.03 (0.83 – 1.27)	0.81	1.05 (0.76 – 1.43)	0.78	50	0.09
	Mixed	1.35 (0.73 – 2.49)	0.33	1.35 (0.73 – 2.49)	0.33	–	–
A1298C (rs1801131)							
Alleles (C vs A)	All	1.10 (0.95 – 1.28)	0.21	1.07 (0.86 – 1.35)	0.54	53	0.05
	Asian	1.18 (0.99 – 1.42)	0.07	1.15 (0.86 – 1.54)	0.35	57	0.08
	Caucasian	0.95 (0.74 – 1.24)	0.72	0.96 (0.66 – 1.40)	0.85	50	0.13
CC vs AA	All	1.62 (1.11 – 2.36)	0.01	1.61 (0.97 – 2.65)	0.06	35	0.16
	Asian	2.00 (1.25 – 3.21)	0.004	2.04 (1.09 – 3.81)	0.03	33	0.22
	Caucasian	1.16 (0.62 – 2.16)	0.65	1.12 (0.51 – 2.48)	0.78	31	0.23
CC vs (CA + AA)	All	1.68 (1.17 – 2.43)	0.005	1.69 (1.09 – 2.64)	0.02	24	0.25
	Asian	2.49 (1.42 – 4.36)	0.001	2.04 (1.13 – 3.70)	0.02	29	0.24
	Caucasian	1.31 (0.72 – 2.39)	0.37	1.28 (0.65 – 2.54)	0.48	17	0.30
(CC + CA) vs AA	All	1.02 (0.85 – 1.23)	0.83	0.98 (0.74 – 1.29)	0.89	51	0.05
	Asian	1.11 (0.89 – 1.38)	0.37	1.05 (0.73 – 1.52)	0.79	59	0.06
	Caucasian	0.85 (0.61 – 1.19)	0.34	0.86 (0.56 – 1.33)	0.50	38	0.20

the allele model. The stratification analysis by ethnicities was of concern, and the results revealed a marginal association in mixed population (homozygous model, OR=3.37, 95% CI=1.01–13.68,

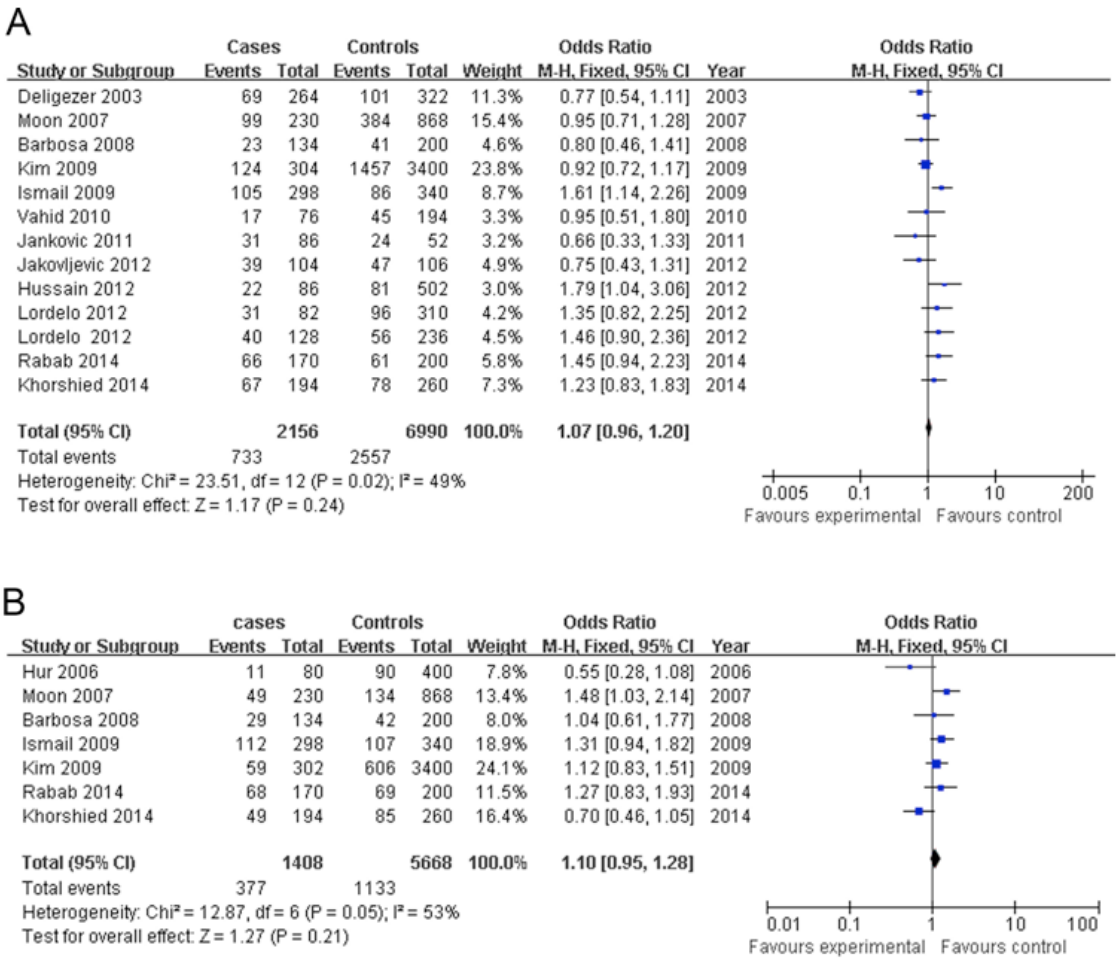

Figure 2. Forest plots of MTHFR polymorphisms and CML risk. **A:** Forest plots of MTHFR C677T polymorphism and risk of CML under the allele comparison model (T vs A); **B:** Forest plots of MTHFR A1298C polymorphism and risk of CML under the allele comparison model (C vs A).

p=0.05; dominant model, OR=3.50, 95% CI=0.98–12.45, p=0.05), but this association lacked statistical power due to its small sample size as only one study was included. For the analysis of MTHFR A1298C polymorphism on the CML risk, the results showed no significant association for the allele model (Figure 2B). However, a significant association was found under the homozygous model (CC vs AA, OR=1.62, 95% CI=1.11–2.36, p=0.01) in the overall population. Subgroup analysis by ethnicity showed a magnitude effect in Asian population under the homozygous model (CC vs AA, OR=2.00, 95% CI=1.25–3.21, p=0.004). Moreover, a positive association under the dominant model (CC vs CA+AA) was found for MTHFR A1298C polymorphism (OR=1.68, 95% CI=1.17–2.43, p=0.005) in the overall population. Stratification estimates of the effect for A1298C on CML risk revealed a magnitude association in Asian population under the dominant model (CC vs CA + AA, OR=2.49, 95% CI=1.42–4.36, p=0.001). Estimation for between-study heterogeneity was performed using Q statistic and I^2 metric, and the results revealed moderate heterogeneities for most of comparisons under different genetic models since the I^2 % values mainly ranged from 25 to 50%. However, large heterogeneities were found in the subgroup analysis for MTHFR C677T polymorphism in Asian population under all comparison models, and for comparisons between MTHFR A1298C polymorphism and CML risk using the allele and recessive models (Table 3).

Publication bias

Beggs' funnel plot and Egger's test were applied to assess possible publication bias of the included studies. The results showed that the sharps

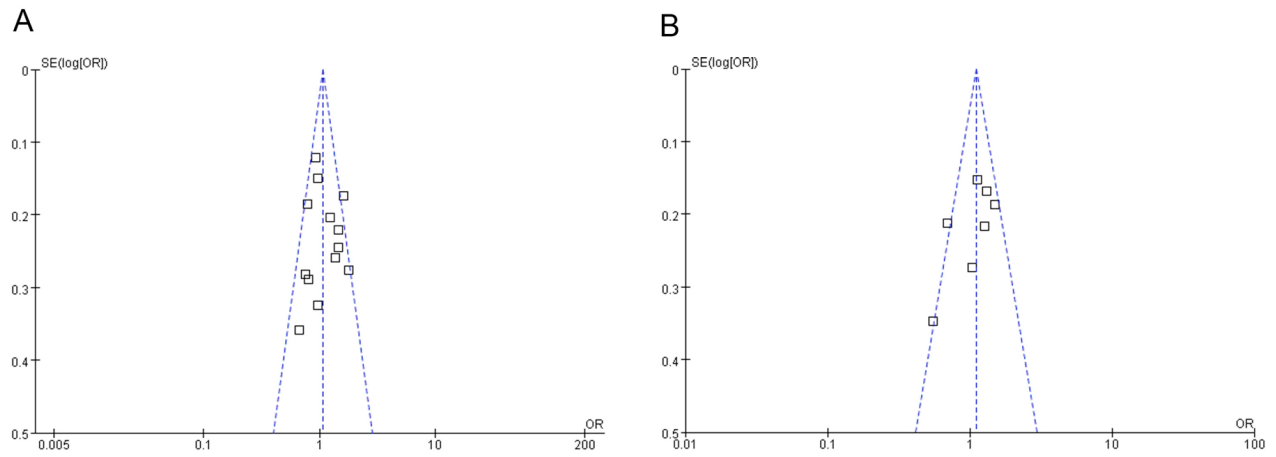

Figure 3. Funnel plots analysis of potential publication bias. **A:** Funnel plots analysis for publication bias between MTHFR C677T polymorphism and CML; **B:** Funnel plots analysis for publication bias between MTHFR A1298C polymorphism and CML.

of the funnel plots were symmetrical in all the comparisons for both C677T and A1298C polymorphism under different genetic models in the overall population and subgroup comparisons by ethnicity (Figure 3 for allele model, other data not shown). Egger's test also didn't reveal significant asymmetry (p=0.741 for MTHFR C677T polymorphism under the allele model, and p=0.166 for MTHFR polymorphism A1298C under the allele model). Finally, there was no obvious evidence showing potential publication bias in this meta-analysis.

Sensitivity analysis

The results of sensitivity analysis showed that no individual study influenced the pooled ORs, indicating the results of this meta-analysis are stable (Figure 4).

Discussion

It is well recognized that individuals' susceptibility to certain kinds of cancer are different, even they were exposed to identical environment. This difference can be explained by the host factors including gene polymorphisms. Therefore, it is reasonable to investigate the possible association between individual's genetic susceptibility and the development of cancer. MTHFR is a pivotal enzyme in the folate metabolism pathway, and studies in past decades have found that two common MTHFR polymorphisms, C677T and A1298C, have possible association with the risk of various kinds of carcinomas, including colorectal cancer [29], lung cancer [30], prostate cancer [31,32], breast cancer [33], gastric cancer [34,35], ovarian cancer [36], cervical cancer [37,38], leukemia [39,40], and so on. Decreased enzyme activities were found in both MTHFR C677T and A1298C variants, and different enzyme activities among individuals may account for one's susceptibility to developing cancer, since the MTHFR activity, determining the fate of folate metabolism which can modulate the DNA hypomethylation level and DNA synthesis efficiency, influences in turn the chromosomal stability and DNA replicating fidelity [9,13-15].

Several studies had investigated the relationship between the MTHFR polymorphisms and CML risk in the past decade. However, inconclusive and even contradictory conclusions were found in these studies, partly because small sample size involved in each study while ethnicity differences could explain such conflicting results [16-28,41]. To get a more comprehensive relationship between the MTHFR C677T/A1298C polymorphisms and the CML risk, we conducted this meta-analysis of available publications investigating the effects of MTHFR C677T and/or A1298C on the risk of CML. Overall, we didn't find a significant association between the CML risk and the MTHFR C677T polymorphism under the allele contrast model and under the homozygous, dominant and recessive models. In regard with stratification analysis of MTHFR C677T pol-

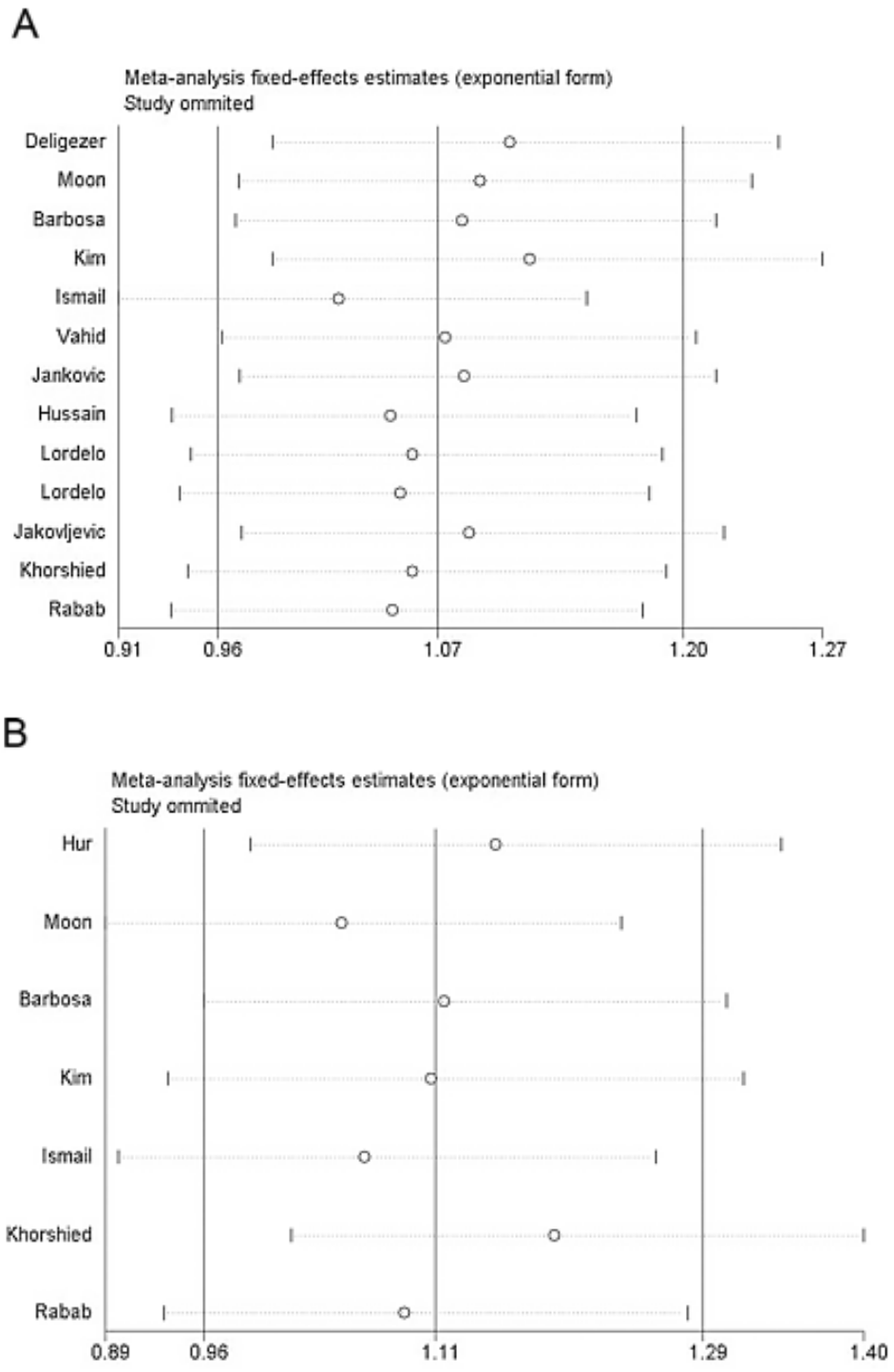

Figure 4. Sensitivity analysis of the relationship between MTHFR polymorphisms C677T (**A**)/ A1298C (**B**) and risk of CML under the allele model.

ymorphism, there were no positive associations under different genetic models as well. The result for MTHFR C677T association analysis was consistent with a previous published meta-analysis by Li et al. [41]. However, significant associations between MTHFR A1298C variants and the CML risk were discovered under the homozygous (CC vs AA) and dominant (CC+AC vs AA) comparison models in the overall population. Moreover, subgroup analysis by ethnicities demonstrated a magnitude association under both homozygous and dominant models between A1298C variants

and CML risk in Asian populations, while no significant effect was found in Caucasian populations, suggesting a possible role of ethnic differences in the genetic background and environmental factors regarding the A1298C variants and the risk of CML. In conclusion, this meta-analysis evidenced that MTHFR 1298C carriers possessed increased susceptibility to development of CML, especially in Asian populations. Contrary to our findings, reduced susceptibility to CML was found in a previously published meta-analysis by Li et al. [41] in terms of the MTHFR A1298C polymorphism. By further analyzing their study, we found that it was improper to include some studies in their analysis because one study [19] for MTHFR C677T polymorphism and two studies [25,28] for MTHFR A1298C polymorphism were mistakenly taken into their analysis, since the genotype distribution of the control group wasn't consistent with the Hardy-Weinberg Equilibrium (PHWE <0.05, Table 2), indicating genotyping error and/or existence of population stratification.

Heterogeneity between studies is a critical problem which should be given high attention. To avoid the potential heterogeneity, carefully publication search, strict studies' inclusion criteria, precise data extraction, and strict statistical analysis were performed in this meta-analysis. However, extreme heterogeneities emerged in comparisons between MTHFR C677T polymorphisms in Asian populations under different genetic models, and in the comparisons between MTHFR A1298C polymorphisms under the allele and recessive models. Heterogeneities may result from selection of the control groups, study design, ethnicity differences, and lifestyle factors, and so on. No substantial publication bias was found by Begg's and Egger's tests.

Considering the limitations of this meta-analysis, the results should be interpreted with caution. Firstly, only publications with full text articles were included in this analysis, thus negative or non-significant results would be unpublished, although no substantial publication bias was found in the analysis. Secondly, the number of available studies in this meta-analysis may be insufficient for a comprehensive understanding of the relationship between MTHFR C677T/A1298C polymorphisms and CML risk. Thus, more case-control studies should be involved in subsequent analyses. Finally, this meta-analysis was conducted by unadjusted estimates, and a more precise analysis should be carried out using individual data if they were available, which would allow the researchers to adjust other covariates including disease phase of CML, folate status, family history, and environmental factors.

In conclusion, although previous studies reached contradictory conclusions in addressing the relationship between MTHFR C677T/A1298C polymorphisms and the risk of CML, the results of this meta-analysis suggested no significant association between MTHFR C677T polymorphism and CML risk and an increased CML risk for 1298C variant carriers, especially more obvious in Asian population. However, large-scale case-control studies and gene-gene or gene-environmental factors should be taken into consideration for subsequent analyses to clarify more precisely the relationship between MTHFR polymorphism and the risk of CML.

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Authors' contributions

Conceived and designed the experiments: LC TY. Performed the experiments: LC YJ JL. Analyzed the data: LC YZ QL. Contributed reagents/materials/analysis tools: LC YJ JL. Wrote the paper: LC. Revised the manuscript: LC TY.

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