RESEARCH ARTICLE

MTHFR C677T Polymorphism and Ovarian Cancer Risk: A Meta-analysis

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Abstract

Background: Many studies have investigated possible association between the methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and ovarian cancer risk, but the impact is still unclear owing to the obvious inconsistencies. This study was performed to quantify the strength of the association with a meta-analysis. Methods: We searched the PubMed, Embase, and CNKI databases for studies relating the association between MTHFR C677T polymorphism and ovarian cancer risk and estimated summary odds ratios (ORs) with confidence intervals (CIs) for assessment. Results: Finally, eight studies with a total of 3,379 ovarian cancer cases and 4,078 controls were included into this meta-analysis. Overall the showed that MTHFR C677T polymorphism was not associated with ovarian cancer risk under all genetic models ($OR_{T \text{ versus } C}$ = 1.03, 95% CI 0.90-1.18; $OR_{TT \text{ versus } CC}$ = 1.08, 95% CI 0.79-1.47; $OR_{TT \text{ versus } TC+CC}$ = 1.05, 95% CI 0.80-1.37; $OR_{TT+TC \text{ versus } CC}$ = 1.05, 95% CI 0.86-1.21). Meta-analyses of studies with confirmation of HWE also showed no significant association. Subgroup analyses by ethnicity showed there was no significant association in the Caucasians but MTHFR C677T polymorphic variant T contributed to increased risk of ovarian cancer in East Asians. No evidence of publication bias was observed. Conclusion: Meta-analyses of available data show that MTHFR C677T polymorphism is not associated with ovarian cancer risk in Caucasians, but the MTHFR polymorphic variant T may contribute to increased risk in East Asians.

Keywords: MTHFR - polymorphism - ovarian cancer - meta-analysis

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Introduction

Ovarian cancer is one of the most common gynecological malignancies with high mortality and it is difficult to make an early diagnosis (Clarke-Pearson, 2009; Jemal et al., 2010). Despite the public health importance of ovarian cancer, its etiology remains unclear (Cannistra, 2004; Pennington and Swisher, 2012). Many studies suggest that the genetic factors play an important role in the etiology of ovarian cancer (Diaz-Padilla et al., 2012; Khanra et al., 2012; Pennington and Swisher, 2012). Those have been analyzed in searching for the molecular basis of ovarian cancer, such as genes mutations in BRCA1 and BRCA2 and mutations in CYP1A2 (Huang et al., 2012; Pennington and Swisher, 2012). Besides, examination of genetic polymorphisms may explain individual differences in risk of ovarian cancer (Bhurgri et al., 2011).

The 5, 10-methylenetetrahydrofolate reductase gene (MTHFR) maps to chromosome 1p36.3, and MTHFR plays a central role in folate metabolism, together with other enzymes by irreversibly catalyzing the conversion of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the primary circulating form of folate and a cosubstrate for homocysteine methylation to methionine (Goyette et al., 1994; Frosst et al., 1995). Many rare mutations of the MTHFR gene have been described in individuals, resulting in very low enzymatic activity, whereas the most common polymorphism is a C to T mutation in exon 4 at nucleotide 677, leading to Ala222Val and presenting in healthy individuals with lower enzyme activity (Goyette et al., 1994; Frosst et al., 1995). This MTHFR genetic polymorphism can lead to abnormal DNA methylation and DNA synthesis, possibly leading to an altered risk for ovarian cancer (Kim, 2005; Dong et al., 2008).

There were many published studies investigating the association between C677T polymorphism and ovarian cancer risk, but the available evidence from the genetic association was still weak, owing to sparseness of data or disagreements among studies (Jakubowska et al., 2007; Terry et al., 2010; Pawlik et al., 2011; Webb et al., 2011; Gao et al., 2012). Small genetic association studies have various designs, different methodology and insufficient power, and could inevitably increase the risk that chance could be responsible for their conclusions, while combining data from all eligible studies by meta-analysis has the advantage of reducing random error and obtaining precise estimates for some potential genetic associations (Petitti, 2000; Attia et al., 2003). We present herein the results of a meta-analysis of published data investigating

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the association between MTHFR C677T polymorphism and ovarian cancer risk to shed some light on these contradictory results and to decrease the uncertainty of the effect size of the estimated risk.

Materials and Methods

Study identification and selection criteria

We searched PubMed, Embase and CNKI database using the following search strategy: ('ovarian carcinoma' or 'ovarian cancer' or 'ovarian tumors' or 'ovary carcinoma' or 'ovary cancer' or 'ovary tumors') and ('Methylenetetrahydrofolate reductase' or 'MTHFR' or 'C677T') and ('polymorphism' or 'polymorphisms' or 'mutation' or 'mutations') for papers published (last search was done on March, 2012). The language of the papers was not restricted. All searched studies were retrieved, and their bibliographies were checked for other relevant publications. When more than one of the same patient population was included in several publications, only the most recent or complete study was used in this meta-analysis. The following criteria were used to select the eligible studies: (1) case-control studies; (2) evaluation of the MTHFR C677T polymorphism and ovarian cancer risk; (3) identification of ovarian cancer was confirmed histologically or pathologically; (4) sufficient reported genotypic frequencies in both case and control populations for estimating an odds ratio (OR) with a 95% confidence interval (CI); (5) the genotype distribution among the control population was consistent with Hardy-Weinberg Equilibrium (HWE). The major reasons for exclusion of studies were: (1) case only studies; (2) review papers; (3) containing overlapping data.

Data extraction

Two investigators independently extracted data, and disagreements were resolved through consensus. The extracted data included the year of publication, ethnicity of the study population, definition of ovarian cancer, inclusion criteria for patients and normal controls, demographics, matching, clinical status of controls, genotyping method, and the genotype distribution of cases and controls for the MTHFR C677T. The frequencies of the alleles were extracted or calculated for cases and controls. All data were extracted from published articles, and we did not contact individual authors for further information.

Statistical analysis

For the control group of each study, the distribution of genotypes was tested for HWE using the Chi-square test (Salanti et al., 2005). The strength of association between MTHFR C677T polymorphism and ovarian cancer risk was estimated by Odds ratios (ORs) with 95% confidence intervals (CIs). Five different comparison models of ORs were calculated: the allele model (T vs. C), the Homozygote comparison model (TT versus CC), the Recessive genetic comparison model (TT versus T/C+CC), and the Dominant genetic comparison model (TT+T/C versus CC). The significance of the pooled OR was determined by the Z test and a p value of less than 0.05 was considered significant. In our study, two models of meta-analysis for dichotomous outcomes were conducted: the random-effects model and the fixed-effects model (Mantel and Haenszel, 1959; DerSimonian and Laird, 1986). The random-effects model was conducted using the DerSimonian and Laird's method, which assumed that studies were taken from populations with varying effect sizes and calculated the study weights both from in-study and between-study variances (DerSimonian and Laird, 1986). The fixed-effects model was conducted using the Mantel-Haenszel's method, which assumed that studies were sampled from populations with the same effect size and made an adjustment to the study weights according to the in-study variance (Mantel and Haenszel, 1959). To assess the between-study heterogeneity more precisely, both the chi-square based Q statistic test (Cochran's Q statistic) to test for heterogeneity and the I^2 statistic to quantify the proportion of the total variation due to heterogeneity were calculated (Cochran, 1954, Higgins et al., 2003). The I^2 index expressing the percentage of the total variation across studies due to heterogeneity was calculated to assess the between-study heterogeneity. I^2 values of 25%, 50%, and 75% were used as evidence of low, moderate, and high heterogeneity, respectively (Higgins et al., 2003). If moderate or high heterogeneity existed, the random-effects model was used to pool the results; otherwise, the fixed-effects model was used to pool the results when I^2 value was less than 50%. To study the source of between-study heterogeneity, meta-regression was also performed (Thompson and Higgins, 2002). To validate the credibility of outcomes in this meta-analysis, sensitivity analysis was performed by sequential omission of individual studies or by omitting studies without high quality (Tobias, 1999). Besides, sensitivity analysis was also performed by adding those excluded studies with controls not in HWE (Salanti et al., 2005).

For additional analyses, the cases and controls were sub-grouped on the basis of their ethnicity. Racial/ethnic descent was categorized into Caucasians, East Asians, and others according to ethnicity classifications for genetic studies (Burchard et al., 2003; Bhopal, 2004). Publication bias was investigated by Begg's funnel plot, in which the standard error of logor of each study was plotted against its logor, and an asymmetric plot suggested possible publication bias (Stuck et al., 1998). In addition, funnel-plot's asymmetry was assessed by the method of Egger's linear regression test (Egger et al., 1997).

All analyses were performed using STATA version 12.0 (StataCorp LP, College Station, Texas). A p value < 0.05 was considered statistically significant, except where otherwise specified.

Results

Characteristics of included studies

With our search criterion, 17 individual records were found, 8 full-text publications were preliminarily identified for further detailed evaluation after excluding 9 records (Gershoni-Baruch et al., 2000; Jakubowska et al., 2007; Wu et al., 2007; Magnowski et al., 2010; Terry et al., 2010; Pawlik et al., 2011; Prasad and Wilkhoo, 2011; Webb et al., 2011). According to the exclusion criteria,

Table 1. Characteristics of Studies on the Association Between the MTHFR C677T Polymorphism and Ovarian Cancer Risk

Author (year)	Ethnicity	Cases	Controls	Genotype frequency (TT:CT:CC)	Genotype method†	P_{HWE}*
Jakubowska et al., 2007	Caucasians	146 patients with ovarian cancer	290 unaffected controls	(case) 15:56:73 (control) 18:134:128	PCR-RFLP	0.03
Wu et al., 2007	East Asians	81 patients with ovarian cancer	80 healthy controls	(case) 24:40:17 (control) 13:35:32	PCR-RFLP	0.52
Terry et al., 2010	Caucasians	1059 patients with ovarian cancer	1125 healthy controls	(case) 140:492:427 (control) 138:488:499	PCR-RFLP	0.27
Terry et al., 2010	Caucasians	153 patients with ovarian cancer	482 non-cancer controls	(case) 10:72:71 (control) 55:217:210	PCR-RFLP	0.93
Terry et al., 2010	Caucasians	364 patients with ovarian cancer	412 non-cancer controls	(case) 33:167:164 (control) 51:168:193	PCR-RFLP	0.13
Webb et al., 2011	Caucasians	1363 patients with ovarian cancer	11414 non-cancer population controls	(case) 154:590:619 (control) 154:628:632	PCR-RFLP	0.91
Prasad and Wilkhoo, 2011	Caucasians	80 patients with ovarian cancer	125 controls	(case) 5:3:72 (control) 1:8:116	PCR-RFLP	0.06
Pawlik et al., 2011	Caucasians	135 patients with ovarian cancer	160 unrelated healthy female volunteers	(case) 13:55:67 (control) 18:79:63	PCR-RFLP	0.36

*P HWE was for the P value of Hardy-Weinberg equilibrium; †PCR-RFLP, PCR restriction fragment length polymorphism

two publications were excluded including one for lack of available data (Gershoni-Baruch et al., 2000) and one for case only study (Magnowski et al., 2010). One paper reported three individual case-control studies, and the data from this paper were extracted as three individual case-control studies (Terry et al., 2010). At last, 8 individual case-control studies with 3379 cases and 4078 controls were included into this meta-analysis (Jakubowska et al., 2007; Wu et al., 2007; Terry et al., 2010; Pawlik et al., 2011; Prasad and Wilkhoo, 2011; Webb et al., 2011). The detailed characteristics of these studies are summarized in Table 1. The number of cases varied from 80 to 1363, with a mean of 422, and the numbers of controls varied from 80 to 1414, with a mean of 510 (Table 1). There were 7 studies with confirmation of HWE, and 1 study with departures from HWE (Table 1).

Main results

Table 2 and Figure 1 show the results for the association between CCND1 G870A polymorphism and ovarian cancer risk. Meta-analyses of total studies showed that MTHFR C677T polymorphism was not associated under all genetic models (OR T versus C = 1.03, 95%CI 0.90-1.18; OR TT versus CC = 1.08, 95%CI 0.79-1.47; OR TT versus TC+CC = 1.05, 95%CI 0.80-1.37; OR TT +TC versus CC = 1.05, 95%CI 0.86-1.21). Meta-analyses of studies with confirmation of HWE also showed no significant association between MTHFR C677T polymorphism and ovarian cancer risk.. Sensitivity analyses by sequential omission of individual studies or by adding those excluded studies with controls not in HWE also did not materially alter the overall combined ORs.

There was obvious heterogeneity for the contrast models (Table 2).The meta-regression showed that the major source of heterogeneity was the ethnicity (P <0 .01). However, other possible sources of heterogeneity were not found. Besides, subgroup by ethnicity further there was no obvious heterogeneity for both subgroup analyses of Caucasians and East Asians (Table 2).

Subgroup analyses by ethnicity showed there was no significant association in the Caucasians but MTHFR C677T polymorphic variant T contributed to increased risk of ovarian cancer in East Asians. In subgroup analysis by ethnicity, the pooled ORs were not significant for all genetic models in Caucasians (Table 2). In East Asians, the pooled ORs were significant under all four genetic

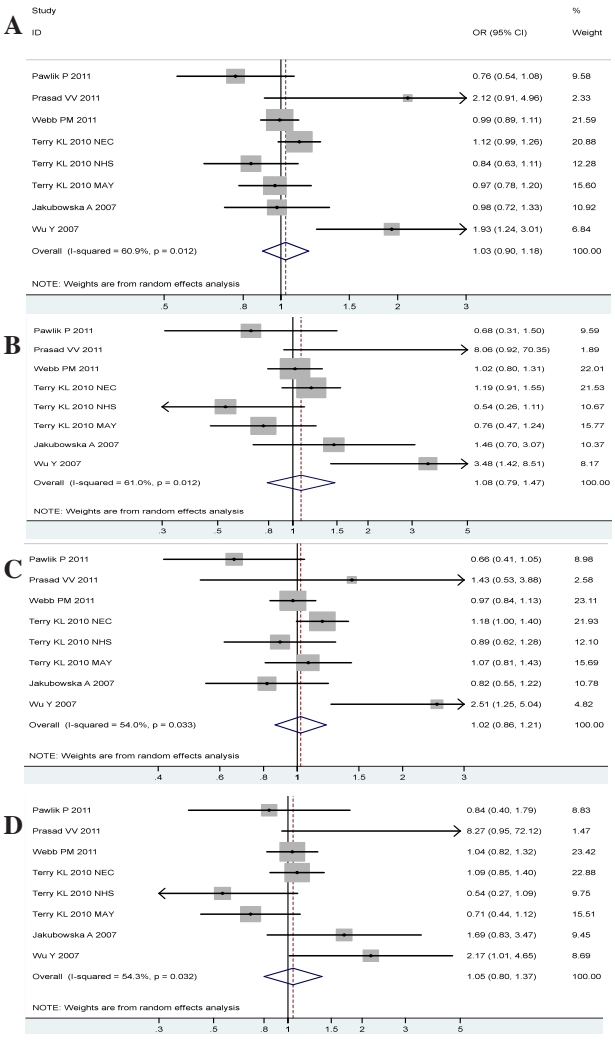

Figure 1. Forest plot for Meta-analysis of the Association Between the MTHFR C677T Polymorphism and Ovarian Cancer Risk (On the left, the first author of the study was followed by the publication year in parentheses. The size of the black box corresponding to each study was proportional to the sample size, and the horizontal line shows the corresponding 95% CI of the odds ratio. The combined estimate was shown by the diamond). 1-A (T versus C, Allele contrast model) 1-B (TT versus CC, Homozygote model) 1-C (TT+CT versus CC, Dominant model) 1-D (TT versus TC+CC, Recessive model)

Table 2. Odds Ratios and Heterogeneity Results for the Meta-analysis of MTHFR C677T Polymorphism and Ovarian Cancer Risk

Comparison Model	OR(95%CI) †	P_{OR}	Model	I^2*
Total studies				
T versus C	1.03(0.90-1.18)	0.684	Random	60.90%
TT versus CC	1.08(0.79-1.47)	0.633	Random	61.00%
TT versus TC+CC	1.05(0.80-1.37)	0.725	Random	54.30%
TT+ TC versus CC	1.02(0.86-1.21)	0.801	Random	54.00%
Studies with confirmation of HWE				
T versus C	1.04(0.89-1.21)	0.633	Random	66.30%
TT versus CC	1.04(0.74-1.47)	0.803	Random	65.10%
TT versus TC+CC	1.00(0.75-1.32)	0.99	Random	55.40%
TT+ TC versus CC	1.05(0.88-1.26)	0.597	Random	56.80%
Caucasian				
T versus C	1.01(0.94-1.08)	0.786	Fixed	39.60%
TT versus CC	1.02(0.87-1.20)	0.783	Fixed	45.50%
TT versus TC+CC	1.01(0.87-1.17)	0.926	Fixed	48.20%
TT+ TC versus CC	1.01(0.92-1.12)	0.76	Fixed	32.30%
Caucasian studies with confirmation of HWE				
T versus C	1.01(0.94-1.09)	0.754	Fixed	49.40%
TT versus CC	0.94(0.71-1.24)	0.652	Random	50.30%
TT versus TC+CC	0.98(0.85-1.15)	0.842	Fixed	47.30%
TT+ TC versus CC	1.03(0.93-1.13)	0.583	Fixed	35.20%
Asian				
T versus C	1.93(1.24-3.01)	0.004	Fixed	---#
TT versus CC	3.48(1.42-8.51)	0.006	Fixed	---#
TT versus TC+CC	2.17(1.01-4.65)	0.046	Fixed	---#
TT+ TC versus CC	2.51(1.25-5.04)	0.01	Fixed	---#

*I^2, the I^2 value of heterogeneity analysis; #Data were not be calculated out; †OR, Odds Ratio; 95%CI, 95% Confidence Interval

models.

Publication bias

Funnel plot and Egger's test were performed to assess the publication bias in this meta-analysis. Funnel plots' shape of all contrasts did not reveal obvious evidence of asymmetry, and all the P values of Egger's tests were more than 0.05, providing statistical evidence of funnel plot symmetry.

Discussion

Due to the different role of MTHFR genetic polymorphism on abnormal DNA methylation and DNA synthesis, it has been hypothesized that MTHFR C677T polymorphism is associated with risk of ovarian cancer, and many reports have been published but no clear consensus has been reached. This led us to undertake the present meta-analysis, which could quantify the synthesis of all the available data and might help us to explore a more robust estimate of the role of this polymorphism with ovarian carcinogenesis. To our knowledge, this is the first meta-analysis on the association between MTHFR C677T polymorphism and ovarian cancer. Eight individual case-control studies with 3379 cases and 4078 controls were included into this meta-analysis. The present research didn't find significant association between MTHFR C677T polymorphism and ovarian cancer in all comparison models. The results from subgroup analyses by ethnicity in Caucasians were similar with that of overall analyses, but there were significant association in East Asians. Up to date, attention has been drawn at a meta-analytical level on the MTHFR C667 polymorphism and potential roles of MTHFR C677T polymorphism have been postulated in various types of cancers (e.g. colorectal, breast, and gastric cancer). The previous meta-analysis demonstrated an increased risk in homozygote carriers of MTHFR 677TT for gastric, breast, and liver caner, but a decreased risk for colorectal cancer (Jin et al., 2009, Taioli et al., 2009, Dong et al., 2010, Zhang et al., 2010). Bedsides, previous meta-analysis also demonstrated there may be ethnicity-based effects of MTHFR C677T polymorphism on the cancer risk (Jin et al., 2009, Taioli et al., 2009, Dong et al., 2010, Zhang et al., 2010, Zhang et al., 2012). The present study finds that MTHFR C677T polymorphism is associated with ovarian cancer risk in East Asians but not in Caucasians, and this difference may come from the ethnicity-specific effects of MTHFR C677T polymorphism on cancer risk

Though the histological subtypes were not uniformly defined in those included studies in this meta-analysis, no subgroup analyses in specific histological subtypes were feasible. As we know, ethnicity, histological and anatomical sites can modulate the effects of gene in cancer susceptibility. Large well-designed cohort studies in the susceptibility of different histological subtypes of ovarian cancer may confirm this association in the future. Both English and Chinese language articles were identified, retrieved and included in the analysis in order to avoid the local literature bias. A limitation still should be acknowledged, in the subgroup analyses, the vast majority of data came from Caucasian populations, the numbers of East Asians were relatively small. The results upon East Asians subjects should be interpreted with caution. As mentioned above, studies on East Asians and other populations are needed to elucidate the possible race-specific effects.

Heterogeneity is a potential problem when interpreting the results of all meta-analyses, and finding of the sources of heterogeneity is one of the most important goals of meta-analysis (Ioannidis et al., 2007). There was obvious heterogeneity for the contrast models (Table 2). The meta-regression showed that the major source of heterogeneity was the ethnicity ($P < 0.01$). However, other possible sources of heterogeneity were not found. Besides, subgroup by ethnicity further there was no obvious heterogeneity for both subgroup analyses of Caucasians and East Asians (Table 2). Thus, the results above suggest ethnicity is the major source of heterogeneity, and there may be race-specific effects of MTHFR C677T polymorphism on cancer risk.

Gene-gene and gene-environmental factors interactions were not fully addressed in this meta-analysis for the lack of sufficient data. Previous studies suggest mutations in BRCA1 and BRCA2 and mutations in CYP1A2 are associated with increased risk of ovarian cancer, and there may be gene-gene interactions. Besides, previous studies suggest folate intake may affect the effects of MTHFR C677T polymorphism on risk of common diseases (Sharp and Little, 2004; Holmes et al., 2011; Kiyohara et al., 2011), and there also be gene-environmental factors interactions in the association between MTHFR C677T polymorphism and ovarian cancer risk. Future studies may further assess the gene-gene and gene-environmental interactions.

In summary, despite the limitations, the results of the present meta-analysis suggest show MTHFR C677T polymorphism is not associated with ovarian cancer risk in Caucasians, but MTHFR polymorphic variant T may contribute to increased risk of ovarian cancer in East Asians. Nevertheless, further larger and well designed studies should be used, which could help us better understanding of the association between this polymorphism and ovarian cancer.

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