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_{TT versus CC} = 1.03, 95%CI 0.90-1.18; OR_{CC versus TC+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. 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...
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 allelle model (T vs. C), the Homozygote comparison model (TT versus CC), the Recessive genetic comparison model (TT versus T/C+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 I² statistic to quantify the proportion of the total variation due to heterogeneity were calculated (Cochran, 1954, Higgins et al., 2003). The I² index expressing the percentage of the total variation across studies due to heterogeneity was calculated to assess the between-study heterogeneity. I² 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 I² 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 Wilkho, 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

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

*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 studies (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 Wilkho, 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 models.
of MTHFR C677T polymorphism have been postulated on the MTHFR C667 polymorphism and potential roles to date, attention has been drawn at a meta-analytical level but there were significant association in East Asians. Up in Caucasians were similar with that of overall analyses, polymorphism and ovarian cancer in all comparison didn’t find significant association between MTHFR C677T included into this meta-analysis. The present research control studies with 3379 cases and 4078 controls were polymorphism and ovarian cancer. Eight individual case-meta-analysis on the association between MTHFR C677T didn’t find significant association between MTHFR C677T polymorphism on cancer risk. 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, 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 suggests 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 that 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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