

Methylenetetrahydrofolate Reductase Gene C677T Polymorphism and Its Association with Ovary Cancer

Vandana Rai

Human Molecular Genetics Laboratory, Department of Biotechnology, VBS Purvanchal University, Jaunpur-222003, India

Abstract

Methylenetetrahydrofolate reductase (MTHFR) is a vital enzyme involved in folate metabolism; a single nucleotide polymorphism (SNP) C677T has been reported to be linked with altered incidences of several diseases. The association between ovary cancer and the MTHFR gene C677T polymorphism has been investigated in several case-control studies. These studies rendered contradictory results, to shed light on these inconclusive findings, a meta-analysis of all available studies relating the C677T polymorphism to the risk of ovary cancer was conducted. The following electronic databases were searched without language restrictions: Pubmed, Google Scholars, Elsevier and Springer Link up to December, 2014. Odds ratios (ORs) with their 95% confidence intervals (95% CIs) were calculated. Meta-analysis was performed using Mix version 1.7.

Eleven studies were finally included in present meta-analysis, which contained 5922 individuals with ovary cancer and 5235 healthy controls. There was not significant relationship between MTHFR C677T polymorphism and ovary cancer under allele contrast (OR: 1.05, 95% CI: 0.99-1.11), dominant (OR: 1.02, 95% CI: 0.91-1.13), recessive (OR: 0.99, 95% CI: 0.90-1.08), homozygous (OR: 0.99, 95% CI: 0.86-1.14) and co-dominant/heterozygous (OR: 1.02, 95% CI: 0.91-1.14) genetic models. Subgroup analysis also reached similar results. Sensitivity analysis indicated that the overall result were dependable.

In conclusion, results of present meta-analysis showed that MTHFR gene C677T polymorphism is not a risk factor for Ovary cancer.

Keywords: Ovary cancer; MTHFR; C677T; Polymorphism; Meta-analysis

Introduction

Cancer is a leading cause of death worldwide. It is estimated that the burden of cancer will increase up to 22.2 million new cases diagnosed annually worldwide by 2030, which represents an increase by 75% Compared with the statistics of 2008 [1,2]. Ovarian cancer is the most common cause of death from gynecological malignancies. In the early stages, women are generally asymptomatic or have non-specific symptoms, making early stage ovarian cancer difficult to diagnose [2-6]. It is the ninth most common malignancy and the fifth most common cause of death from female cancers in the United States [6].

The wide geographic variation in incidence rates points to the role of genetic and environmental factors in the pathogenesis of this cancer. Possible risk factors for ovarian cancer include family history, tobacco smoking, infertility, low parity, and hormone replacement therapy, while oral contraceptive use and fewer menstrual cycles are associated with decreased risk [7-9]. Deficiency of nutrients, such as vitamins and microelements, has also been associated with increased risk for ovarian cancer, whereas high fruit and vegetable intake may help prevent the disease [9,10]. The incidence rate varies geographic worldwide. The wide geographic variation at international levels of ovarian cancer in terms of incidence and mortality suggested the role of genetic and environmental factors in the pathogenesis of this cancer [11].

Folate has a key role in DNA synthesis and methylation, thus, adequate intake is essential to maintain DNA integrity, and its deficiency may increase the risk of mutation, and hence cancer, therefore many countries have introduced mandatory or voluntary fortification of grain products with folic acid. Low folate intake has been associated with an increased risk of several cancers, including breast, and endometrial cancer [8,12-14]. Its influence on ovarian cancer risk is less clear [13,15-17] and may depend on other unmeasured factors

such as genetic variation in the folate metabolism pathway [18]. Folate availability for DNA synthesis and as a methyl donor for methylation depends not only on intake, but also on the activity of enzymes, including methylene tetrahydrofolate reductase (MTHFR), methionine synthase (MTR) and MTR reductase (MTRR) [18,19].

Methylenetetrahydrofolate reductase (MTHFR) is the enzyme responsible for the reduction of methylenetetrahydrofolate. Reduced MTHFR activity results in an increased requirement for folic acid to maintain normal homocysteine remethylation to methionine. In the absence of sufficient folic acid, intracellular homocysteine accumulates, methionine resynthesis is reduced and remethylation reactions are interrupted. Increased homocysteine and decreased methionine cause decreased SAM to S-adenosylhomocysteine (SAH) ratio, which takes part in methylation [20]. There are more than 40 polymorphisms reported in MTHFR gene and among them C677T variant is the most studied and clinically important. The C677T variant (rs 1801133; Ala 222 Val) has been associated with a decreased activity of MTHFR, and increased homocysteine level [21-23]. Mutant homozygous (TT) individuals have a decreased enzymatic activity ~70% and the heterozygote by 40%. A dysfunctional MTHFR leads to lower levels of SAM resulting into DNA hypomethylation. DNA hypomethylation increases the risk of many diseases and disorders like-neural tube

***Corresponding author:** Vandana Rai, Human Molecular Genetics Laboratory, Department of Biotechnology, VBS Purvanchal University, Jaunpur-222003, India, Tel: 05452-252222; Fax: 05452-252244; E-mail: raivandana@rediffmail.com

Received January 06, 2016; **Accepted** May 07, 2016; **Published** May 14, 2016

Citation: Rai V (2016) Methylenetetrahydrofolate Reductase Gene C677T Polymorphism and Its Association with Ovary Cancer. J Health Med Informat 7: 226. doi:10.4172/2157-7420.1000226

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defects [24], cleft lip and palate [25], cardiovascular diseases [21], diabetes [26] and cancer [27] etc.

The different investigations reported the effect of MTHFR C677T polymorphism on ovarian cancer risk in different ethnicities may be due to differences in population background, study design, sample size, environmental factors, and chance variations. Further confirmation of such differential effects is therefore needed. Hence a meta-analysis was carried out to come to a conclusive result whether C677T polymorphism is a risk factor for ovary or not.

Methods

Selection of studies

Electronic searches were conducted using PubMed, Google Scholar, Elsevier and Springer link and all published manuscripts up to March, 2014 were considered in present meta-analysis. The following index terms were used for search 'MTHFR' 'Methylenetetrahydrofolate reductase', and 'C677T polymorphism', and 'Ovary cancer'. In addition, bibliographies of all articles and reviews were hand searched for additional suitable studies.

Data extraction

The following information was extracted from each eligible study: first author's name, journal name, year of publication, country name, number of cases and controls and genotyping method. Number of alleles or genotypes in both cases and controls were extracted or calculated from published data to recalculate ORs.

Inclusion and exclusion criteria

The inclusion criteria of studies were as follow: studies should: 1) Be original, 2) Used case control approach 3) Used PCR-RFLP method for MTHFR genotype analysis and 4) Published in a peer-reviewed journal. Studies were excluded if: 1) Their sample was not independent from that investigated in another study, 2) Incomplete raw data information and not providing complete information for number of allele and/or genotype calculation, 3) Studies based on pedigree data were excluded as they investigate linkage and not association.

Statistical analysis

The present meta-analysis examined the overall association of mutant T allele with the risk of ovary cancer relative to the C allele. The associations were indicated as odds ratios (ORs) with the corresponding 95% CI. A pooled OR was then estimated on the basis of the individual ORs. The OR was estimated either by using fixed effect [28] or random effect [29] models depending upon heterogeneity. When there is considerable heterogeneity between studies then the pooled OR is preferably estimated using the RE model [30]. Models were chosen based on the method described by Thakkinstian et al. [31], briefly calculating and comparing the ORs of T vs. C (allele contrast/additive), TT vs. CC (homozygote), CT vs. CC (co-dominant), TT+CT vs. CC (dominant) and TT vs. CT+CC (recessive) checking the heterogeneity and significance, then determining the best model.

The heterogeneity between studies was tested using the Q-statistic and heterogeneity between studies was quantified using the I^2 statistic [32,33]. If $I^2 > 50\%$ then random effect model was used (which gives wider confidence intervals) otherwise fixed effect model applied. Cumulative meta-analysis was also performed to observe the effect of subsequent addition of each study. Sensitivity analysis was performed to explore potential heterogeneity and verify the stability and robustness of the main results. Quality of each study was assessed using

methodological quality assessment scale according to the methods of Guo et al. [34]. Five major components were judged like: cases diagnosis, source of controls, sample size, quality control of genotyping methods and Hardy-Weinberg equilibrium assessment in controls.

Publication bias

Publication bias was investigated by using funnel plots; viz. funnel plot of standard error by log odds ratio and funnel plot of precision (1/standard error) by log odds ratio. Different statistical tests such as Begg and Mazumdar rank correlation [35] and Egger's regression intercept [36] were adopted to assess the publication bias. All p values are two tailed with a significance level at 0.05. All statistical analyses were undertaken using the freely available program MIX version 1.7 [37].

Results

Eligible studies

Following these exclusions, 9 individual case-control studies with a total of 5,922 cases and 5,235 controls were included into this meta-analysis [9,11,18,19,38-42]. One author collected case samples from three different centers (New England Case Control Study (NEC), Nurses' Health Study (NHS), and Mayo Clinic Ovarian Cancer Case Control Study (MAY)) and reported separately in article, so they were included as separate three studies. Hence, total numbers of 11 studies were included in the present meta-analysis. Three studies investigated Asian population [9,11,40] and other studies investigated Caucasian population [18,19,38,39,41,42].

Characteristics of included studies

In total 11 studies, total cases were 5,922 with CC (3032), CT (2308) and TT (582), and controls were 5,235 with CC (2511), CT (2176), and TT (548). In controls genotypes percentage of CC, CT and TT were 47.97%, 41.57% and 10.47% respectively. In total cases genotype percentage of CC, CT and TT was 51.2%, 39% and 9.8% respectively. Frequencies of CC and CT genotypes were highest in both cases and controls (Table 1). Number of C and T alleles were also calculated and presented in Table 2. Five studies did not show any association (Jakubowska et al.; Terry et al.; Webb et al.; Pawlik et al.) and odds ratio was above one in other studies [18,19,38,39,42].

Meta-analysis:

The main analysis for investigating the association of the C677T allele T and the risk of developing ovary cancer relative to the allele C showed higher heterogeneity ($P=0.0009$, $I^2=65.2\%$) between the 11 studies; the fixed and random pooled OR did not significantly associated with ovary cancer (OR=1.04, 95% CI=0.99-1.11, $p=0.90$) and (OR=1.08, 95% CI=0.95-1.22, $p=0.15$).

The genotype differences for the homozygotes (TT vs. CC) revealed moderate heterogeneity ($P=0.0002$, $I^2=69.55\%$) and did not show any significant association either with fixed effect (OR=0.99, 95% CI=0.86-1.14, $p=0.90$) or random effect model (OR=1.02, 95% CI=0.75-1.37, $p=0.91$). Similarly, genotype contrast using co-dominant, dominant and recessive models also did not show any significant association (CT vs. CC OR=1.01, 95% CI=0.85-1.20, $p=0.90$; TT+CT vs. CC OR=1.01, 95% CI=0.83-1.21, $p=0.93$; TT vs. CT+CC OR=1.03, 95% CI=0.85-1.23, $p=0.76$).

Statistical analysis

In allele contrast meta-analysis, sensitivity analysis performed by exclusion of the studies in which control population was not in Hardy Weinberg equilibrium, studies with small sample size (<100) and

Study	Ethnicity	Case	Control	References
Jakubowska et al. [38]	Caucasian	144	280	Breast Cancer Res Treat 104: 299-308
Wu et al. [40]	Asian	81	80	Xian Dai Fu Chan Ke Jin Zhan 16: 811-813
Terry (NEC) et al. [18]	Caucasian	1059	1125	Gynecol Oncol 119: 319-324
Terry (NHS) et al. [18]	Caucasian	153	482	Gynecol Oncol 119: 319-324
Terry (MAY) et al. [18]	Caucasian	364	412	Gynecol Oncol 119: 319-324
Webb [19]	Caucasian	1668	1278	Eur J Clin Nutr 65: 1133-1140
Prasad and Wilkhoo [41]	Caucasian	80	125	Onkologie 34: 422-426
Gao et al. [11]	Asian	224	432	Asian Pac J Cancer Prev 13: 569-573
Jakubowska et al. [38]	Caucasian	1664	483	Br J Cancer 106: 2016-2024
Pawlik et al. [42]	Caucasian	135	160	Mol Biol Rep 39: 5553-5560
Zhang et al. [9]	Asian	215	218	Int J Mol Sci 13: 4009-4020

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

Study	CC genotype		CT genotype		TT genotype		C allele		T allele	
	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
Jakubowska et al. [38]	73	128	56	134	15	18	202	390	86	170
Wu et al. [40]	17	32	40	35	24	13	74	99	88	61
Terry (NEC) et al. [18]	427	499	492	488	140	138	1346	1486	772	764
Terry (NHS) et al. [18]	71	210	72	217	10	55	214	637	92	327
Terry (MAY) et al. [18]	164	193	167	168	33	51	495	554	233	270
Webb [19]	774	571	709	568	185	139	2257	1710	1079	846
Prasad and Wilkhoo [41]	72	116	3	8	5	1	147	240	13	10
Gao et al. [11]	97	232	100	178	27	22	294	642	154	222
Jakubowska et al. [38]	1155	334	465	130	44	19	2775	798	553	168
Pawlik et al. [42]	67	63	55	79	13	18	189	205	81	115
Zhang et al. [9]	102	115	94	92	19	11	298	322	132	114

Table 2: The distribution of MTHFR C677T genotypes and allele numbers in ovary cancer cases and controls.

studies with high p values. Control population of one study [38] was not in HW equilibrium and heterogeneity did not decreased after exclusion of that study ($p=0.007$, $I^2=65.99\%$). Exclusion of one study with small sample size, less than 100 [41], also did not decreased heterogeneity ($P_{\text{heterogeneity}}=0.001$, $I^2=65.49\%$). Similarly exclusion of five studies with very high p value [18,19,38,39] did not decrease heterogeneity ($P_{\text{heterogeneity}}=0.007$, $I^2=65.99\%$) but increased odds ratio (OR=1.27, 95% CI=1.04-1.51 $p=0.02$) and showed significant association between C677T polymorphism and ovary cancer with allele contrast model (Figures 1 and 2).

Publication bias

Funnel plots, Begg's and Egger's test were performed to estimate the risk of publication bias. The shape of funnel plots in all contrast models showed obvious evidence of symmetry (Figure 3). In addition, all the P values of Egger's test were more than 0.05, which provided statistical evidence for the symmetry of funnel plots in the meta-analysis ($p=0.24$ for T vs. C; $p=0.95$ for TT vs. CC; and $p=0.29$ for CT vs. CC; $p=0.46$ for TT+CT vs. CC; $p=0.64$ for TT vs. CT+CC) (Table 3). Begg's test results also did not show publication bias ($p=0.29$ for T vs. C; $p=0.49$ for TT vs. CC; and $p=0.24$ for CT vs. CC; $p=0.24$ for TT+CT vs. CC; $p=0.54$ for TT vs. CT+CC) (Table 4).

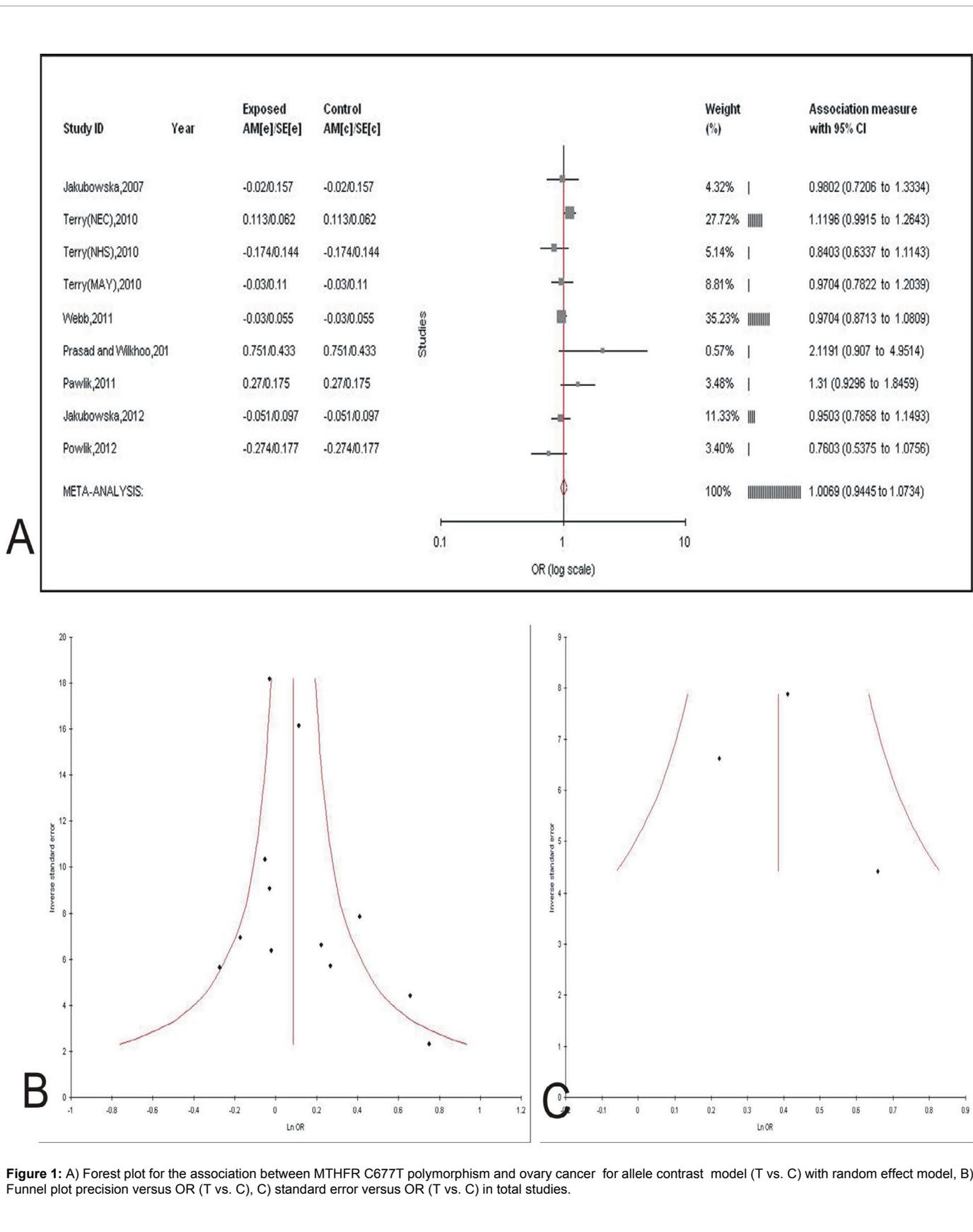
Discussion

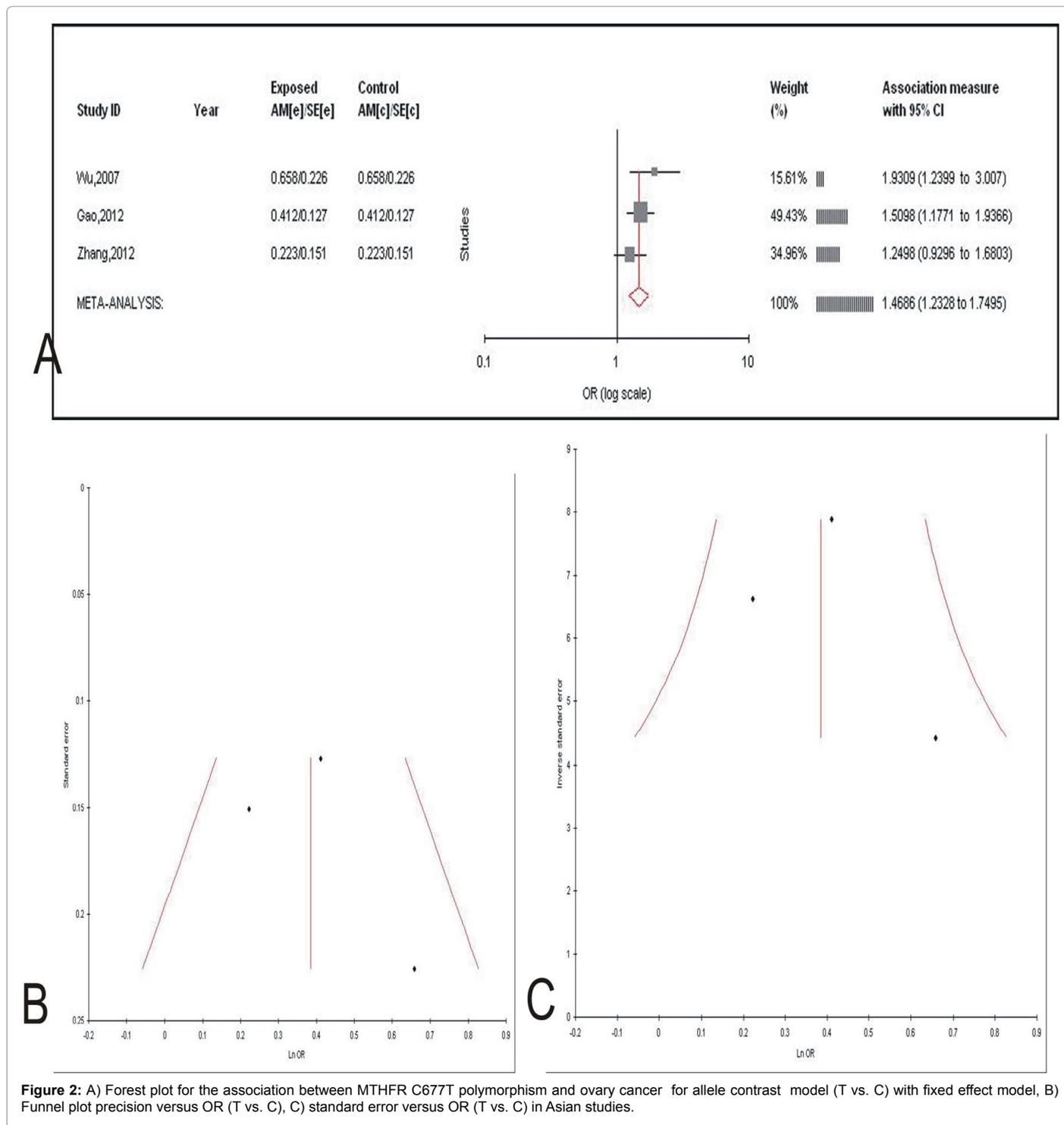
Present meta-analysis of the association of the MTHFR C677T polymorphism with ovary cancer investigated 5,922 patients and 5,235 controls from 11 case-control studies. Overall meta-analysis did not detect significant genetic association between the MTHFR C677T polymorphism and ovarian cancer.

Folate, methionine, vitamin B6 and vitamin B12 may influence carcinogenesis due to their roles in the one-carbon metabolism pathway

which is critical for DNA synthesis, methylation and repair. However, DNA synthesis is also integral to the process of tumor formation, and for many years anti-folates have been used to treat some cancers. Recent studies have suggested that rather than preventing cancer, high folate levels might promote progression of pre-neoplastic lesions to cancer (Table 5) [19], and that the introduction of mandatory folate fortification in Canada and the United States of America may have led to increases in colorectal cancer rates [19].

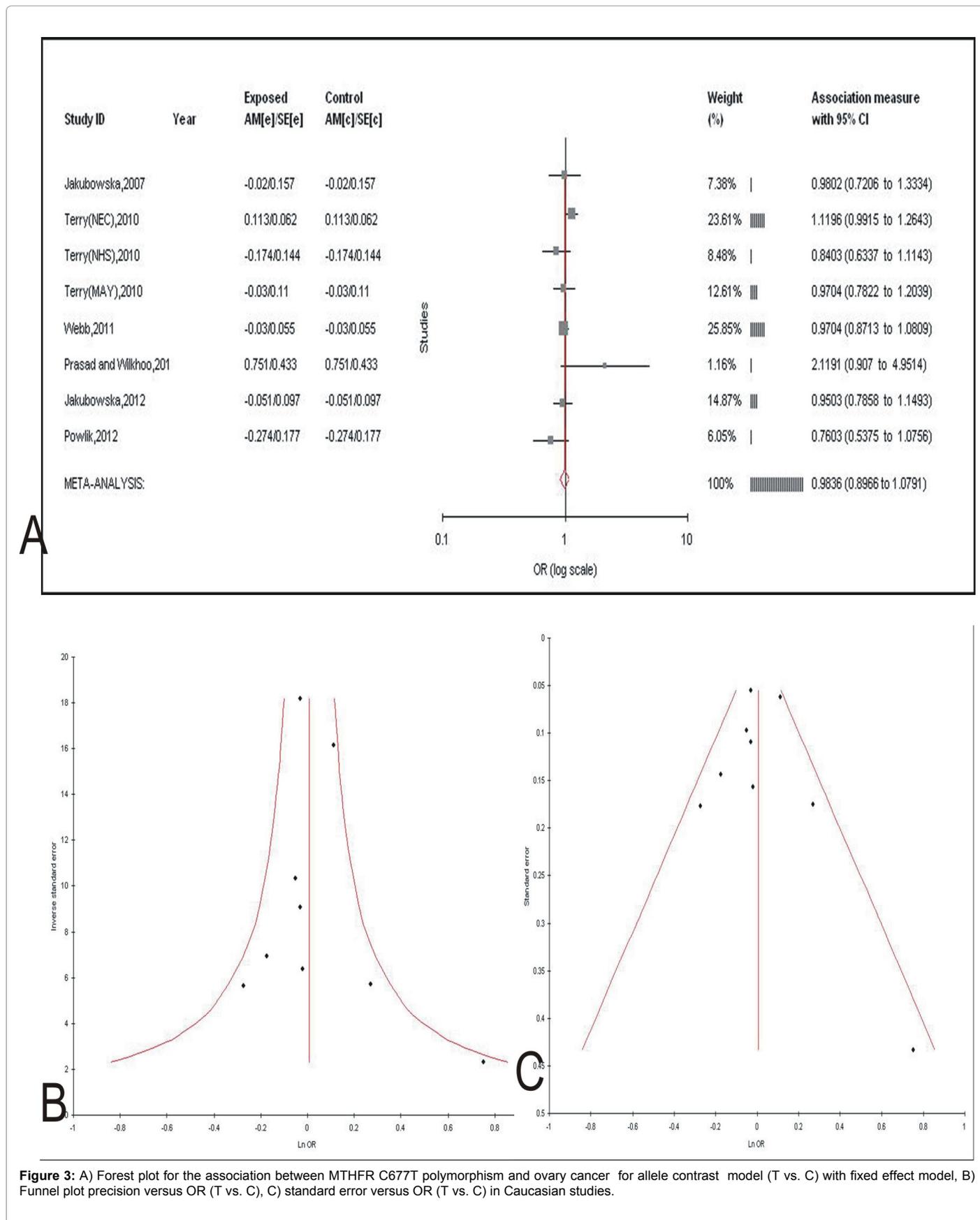
Epidemiologic studies have revealed that MTHFR polymorphisms are associated with an increased risk of esophageal cancer, gastric cancer, breast cancer, hepatocellular carcinoma, bladder cancer, and prostate cancer. Conversely, MTHFR polymorphisms have also been associated with a reduced risk of colon cancer, leukemia, and highly aggressive prostate cancer [43-46]. MTHFR plays a central role in balancing DNA synthesis (which involves 5,10-methylenetetrahydrofolate) and DNA methylation (which involves 5,10-methyltetrahydrofolate). Specifically, the 677T allele contributes to DNA hypomethylation, which in turn may lead to altered gene expression. This polymorphism might exert a protective effect, as observed for colorectal cancer [47], by increasing the levels of the MTHFR substrate, essential for DNA synthesis. The substrate of MTHFR enzyme, 5,10-methylenetetrahydrofolate, is involved in the conversion of deoxyuridylate monophosphate to deoxythymidylate monophosphate, and low levels of 5,10-methylenetetrahydrofolate would lead to an increased deoxyuridylate monophosphate/deoxythymidylate monophosphate ratio. In this situation, increased incorporation of uracil into DNA in place of thymine may follow, resulting in an increased chance of point mutations and DNA/chromosome breakage [48]. A less active form of MTHFR would lead, all other factors being equal, to an accumulation of 5,10-methylenetetrahydrofolate, thus a lower deoxyuridylate monophosphate/deoxythymidylate monophosphate ratio, and a presumably lower cancer risk [48].





Meta-analysis provides a standardized approach for examining the existing literature on a specific, possibly controversial, issue to determine whether a conclusion can be reached regarding the effect of a polymorphism of low penetrant gene [49]. Several meta-analyses were published to assess the role of MTHFR polymorphism in cancer development like: breast cancer [50,51] lung cancer [52] colorectal cancer [53] pancreatic cancer [54] Esophageal cancer [55,56] and cervical cancer [57].

There were several limitations in present study: 1) Crude ORs was used in the pooled analysis without adjustment; 2) The robustness of every single study would be affected by the technique they used; 3) The relatively small sample sizes of some studies is included in the analysis; 4) Meta-analysis was restricted on only single polymorphism, other polymorphism of folate pathway genes should also be included in future meta-analysis and 5) Except one genetic polymorphism, other important factors such as age, ethnicity, and folate intake, and



Genetic Models	Fixed effect	Random effect	Heterogeneity p-value (Q test)	I ² (%)	Publication Bias (p of Egger's test)
	OR (95% CI), p	OR (95% CI), p			
Allele Contrast (T vs. C)	1.04 (0.99-1.11), 0.90	1.08 (0.95-1.22), 0.15	0.0009	66.2	0.32
Co-dominant (CT vs. CC)	1.02 (0.91-1.14), 0.68	1.01 (0.85-1.20), 0.90	0.03	46.9	0.29
Homozygote (TT vs. CC)	0.99 (0.86-1.14), 0.90	1.02 (0.75-1.37), 0.91	0.0002	69.55	0.95
Dominant (TT+CT vs. CC)	1.02 (0.91-1.13), 0.75	1.01 (0.83-1.21), 0.93	0.006	57.96	0.46
Recessive (TT vs. CT+CC)	0.99 (0.90-1.08), 0.79	1.03 (0.85-1.23), 0.76	0.001	63.42	0.64

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), and the I² metric and publication bias p-value (Egger test) in total studies.

Genetic Models	Fixed effect OR (95% CI), p	Random effect OR (95% CI), p	Heterogeneity p-value (Q test)	I ² (%)	Publication Bias (p of Egger's test)
Allele Contrast (T vs. C)	1.47 (1.23-1.74), <0.0001	1.48 (1.20-1.81), 0.0002	0.26	24.68	0.61
Co-dominant (Ct vs. CC)	1.18 (0.92-1.5), 0.19	1.14 (0.82-1.56), 0.42	0.22	33.11	0.10
Homozygote (TT vs. CC)	1.46 (0.97-2.19), 0.06	1.21 (0.33-4.54), 0.77	0.0001	88.99	0.32
Dominant (TT+CT vs. CC)	1.25 (0.99-1.57), 0.06	1.1 (0.64-1.8), 0.8	0.02	74.93	0.06
Recessive (TT vs. CT+CC)	1.28 (0.88-1.85), 0.2	1.24 (0.39-3.87), 0.7	0.0002	88.16	0.72

Table 4: 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), and the I² metric and publication bias p-value (Egger test) in Asian population.

Genetic models	Fixed effect OR (95% CI), p	Random effect OR (95% CI), p	Heterogeneity p-value (Q test)	I ² (%)	Publication Bias (p of Egger's test)
Allele Contrast (T vs. C)	1.001 (0.94-1.07), 0.83	0.99 (0.90-1.09), 0.98	0.10	39.33	0.87
Co-dominant (Ct vs. CC)	0.99 (0.87-1.11), 0.83	0.97 (0.79-1.19), 0.78	0.04	50.28	0.41
Homozygote (TT vs. CC)	0.94 (0.81-1.09), 0.42	0.95 (0.75-1.18), 0.63	0.10	40.03	0.71
Dominant (TT+CT vs. CC)	0.96 (0.86-1.05), 0.55	0.96 (0.8-1.16), 0.7	0.07	44.27	0.64
Recessive (TT vs. CT+CC)	0.97 (0.88-1.06), 0.5	0.98 (0.86-1.11), 0.76	0.20	27	0.90

Table 5: 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), and the I² metric and publication bias p-value (Egger test) in Caucasian population.

smoking status were not considered. Present meta-analysis had several strength also like- (i) The publication bias was not detected in present meta-analysis, (ii) Pooled number of cases and controls from different studies significantly increased the statistical power of the analysis, (iii) Distribution of genotypes in controls except one study was in Hardy-Weinberg equilibrium.

In summary, the results of current meta-analysis indicated a lack of association between MTHFR C677T gene polymorphism and ovary cancer. The results of the present meta-analysis were based on relatively small numbers of studies, participants and higher between studies heterogeneity, hence must be interpreted with caution.

Acknowledgment

The authoress is highly grateful to Leon Bax (Chief Scientific Officer at BiostatXL, UMC Utrecht) for his valuable suggestions, which help her in statistical analysis.

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