Association between MTHFR C677T, MTHFR A1298C and MS A2756G polymorphisms and risk of cervical intraepithelial neoplasia II/III and cervical cancer: A meta-analysis

JIE ZHU[1*], LEI WU[2*], MARTIN KOHLMEIER[3], FANGLI YE[2] and WEI CAI[1]

[1]Xin Hua Hospital, Shanghai Institute for Pediatric Research, School of Medicine, Shanghai Jiaotong University, Shanghai 200092; [2]Medical College, Wuhan University of Science and Technology, Wuhan, Hubei 430065, P.R. China; [3]Nutrigenetics Laboratory, UNC Nutrition Research Institute, University of North Carolina at Chapel Hill, Kannapolis, NC 28081, USA

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Abstract. Numerous case-control studies on the association between polymorphisms of key genes involved in methionine remethylation [methylenetetrahydrofolate reductase (MTHFR) and methionine synthase (MS)] and the susceptibility of cervical intraepithelial neoplasia (CIN) and cervical cancer have provided inconclusive results. The aim of the present meta-analysis was to determine the effects of two MTHFR (C677T and A1298C) and one MS gene polymorphism (A2756G) on the risk of CIN II/III or cervical cancer. Relevant data were retrieved following a systematic search in PubMed, Web of Science, MEDLINE and Wanfang Data up to November 2012. Pooled odds ratios (ORs) with 95% confidence intervals (CIs) were estimated from eligible studies by meta-analysis with subgroup analyses stratified by ethnicity. A total of 13 studies with 1,936 cases and 2,858 controls were included in the present meta-analysis. An increased risk of cervical cancer was found in Asian women with the MTHFR 677T allele (TT vs. CC: OR=1.41, 95% CI=1.07-1.86, P=0.01; TT vs. CC+CT: OR=1.38, 95% CI=1.08-1.75, P=0.008), while a decreased risk was observed in Caucasian women (TT vs. CC: OR=0.65, 95% CI=0.45-0.93, P=0.02; TT+CT vs. CC: OR=0.7, 95% CI=0.58-0.86, P=0.0005). No effects of MTHFR C677T polymorphism on CIN II/III risk and MTHFR A1298C or MS A2756G polymorphisms on cervical cancer risk were detected. The sensitivity analysis suggested stability of this meta-analysis and no publication bias was detected. The MTHFR 677T allele may enhance the risk of cervical cancer in the Asian female population and play a protective role in Caucasian females. However, limited association is suggested between MTHFR A1298C and MS A2756G polymorphisms with cervical tumorigenesis.

Correspondence to: Dr Jie Zhu, Xin Hua Hospital, Shanghai Institute for Pediatric Research, School of Medicine, Shanghai Jiaotong University, 1665 Kongjiang Road, Shanghai 200092, P.R. China
E-mail: jacky284868@163. com

*Contributed equally

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Introduction

Cervical cancer represents the third most common type of cancer in females worldwide with ~500,000 new cases/year. Cervical cancer leads to an estimated 274,000 deaths globally every year, resulting in an increased health and economic burden, particularly in developing countries (1,2). The risk for the development of cervical cancer is enhanced through infection by human papillomavirus (HPV). However, infection with HPV alone is not sufficient for the development of this type of cancer, since several additional host factors may affect the persistence of HPV infection, which induces the malignant conversion of cervical epithelial cells (3-8). DNA hypomethylation has been shown to facilitate the integration of HPV DNA into cells and to reduce the inhibition of HPV expression (3). As a result, enzymes in the one-carbon pathway have received increasing interest since differences in metabolic properties may affect cancer risk.

Methylenetetrahydrofolate reductase (MTHFR) and methionine synthase (MS) are two important enzymes essential for nucleic acid synthesis, DNA repair and methylation; therefore, the investigation of their role in folate metabolic pathways has attracted increased interest. Two common mutations in the MTHFR gene are C677T and A1298C, and the common variant in MS is an A-to-G transversion at position 2756 (MS A2756G) (9,10). Genetic variants of MTHFR and MS genes modify the activities or other kinetic properties of the encoded enzymes (11). The functional consequences of variant enzyme properties may include abnormalities in DNA synthesis, repair and methylation, and, thereby, altered susceptibility to precancerous lesions and cervical cancer (11).

However, controversy remains concerning the role of these polymorphisms in cervical carcinogenesis in terms of

cancer sites, racial differences and the combined influences of additional risk factors (1,11-18). To address such questions, we performed a meta-analysis of published studies to determine potential associations between MTHFR (C677T and A1298C) and MS gene polymorphisms (A2756G) with the risk of CIN II/III and cervical cancer.

Materials and methods

Search strategy and study identification. The present meta-analysis was conducted according to Meta-analysis of Observational Studies in Epidemiology (MOOSE) criteria (19). A literature search for all studies examining the association between the polymorphisms of MTHFR C677T, MTHFR A1298C and MS A2756G with CIN II/III and cervical cancer was performed using electronic databases, including PubMed, Web of Science, MEDLINE and Wanfang Data. The following keywords and subject terms were used: 'cervical intraepithelial neoplasia', 'cervical cancer', 'methylenetetrahydrofolate reductase', 'polymorphism', 'variant', 'mutation', 'folate' and 'one-carbon metabolism' up to November 30th, 2012. 'Methionine synthase' was used to replace 'methylenetetrahydrofolate reductase' in further searches of related studies. References in the identified publications were evaluated and literature retrieval was conducted in triplicate by three independent reviewers (Jie Zhu, Wei Cai and Fangli Ye).

Selection criteria. Eligible studies were included in the present meta-analysis when the following criteria were met: i) the study was an unrelated case-control study examining the association between MTHFR or MS gene polymorphisms and CIN II/III or cervical cancer; ii) the sample size, distribution of genotype frequency or additional information was available; iii) the genotype distribution of the population met all the expectation of the Hardy-Weinberg equilibrium (HWE) theory; iv) for studies where the same or overlapping data were used, the most recent study was included in the present meta-analysis; and v) only studies published as full length articles or letters with adequate study details were used.

Data extraction. Data were collected for meta-analysis according to the selection criteria. The collected information included the year of publication, country, ethnicity, mean age of study population, study design, study method, sample size, source of controls, as well as allele and genotype frequencies in case and control groups.

Statistical analysis. Deviation from HWE was determined by Fisher's exact test in the control group of each study. Crude odds ratios (ORs) with their 95% confidence intervals (CIs) were applied to evaluate the strength of association of MTHFR C677T, MTHFR A1298C and MS A2756G polymorphisms with CIN II/III or cervical cancer, respectively. The pooled ORs and their 95% CIs were calculated and compared for different genetic models for MTHFR C677T allele T [allele model (T vs. C), homozygote model (TT vs. CC), dominant model (TT+CT vs. CC) and recessive model (TT vs. CT+CC)]. The same comparisons were performed for allele C of the MTHFR A1298C and allele G of the MS A2756G polymorphism, respectively.

A Chi-square-based Q-test was conducted to assess heterogeneity between studies, which was considered significant when $P<0.05$. The percentage variability of the overall OR attributable to heterogeneity between studies was assessed by I^2 test. A fixed effects model was used to calculate the summary OR value when heterogeneity did not exist (20). Otherwise, a random effects model (Mantel-Haenszel method) was adopted (20). A Z-test was implemented to determine the significance of the pooled OR and $P<0.05$ was considered to indicate a statistically significant difference. In order to estimate ethnic-specific OR, subgroup analyses were also performed for Asian and Caucasian populations, respectively. The MTHFR A1298C and MS A2756G comparisons for the association with CIN II/III were not stratified for subgroup analysis due to the limitations of the available data. Sensitivity analyses were conducted by reassessing the significance of ORs after each study was omitted in turn. Publication bias was examined with Egger's linear regression test and Begg's funnel plot test.

All statistical analyses were performed using the program Review Manager 5 and STATA software package (version 11.0; StataCorp, College Station, TX, USA). All the P-values were two-sided and $P<0.05$ for any test or model was considered to indicate a statistically significant difference.

Results

Selection of eligible studies. Concerning cervical cancer, 22 studies were retrieved for cervical cancer and 13 met our inclusion criteria; 8 studies were excluded since detailed genotyping information was not available. Moreover, 1 study (8) was replaced with its updated version since the subjects in these 2 studies were from the same population. The final pool of eligible studies consisted of 13 studies with 1,936 cases and 2,858 controls (3,6,12,13,15,17,21-27) for MTHFR C677T polymorphism (Table I), 5 studies with 585 cases and 1,000 controls for MTHFR A1298C polymorphism (Table I), and 3 studies with 389 cases and 440 controls for MS A2756G polymorphism (Table I). The genotype distribution in the controls of all these studies was consistent with HWE. However, not all the studies provided enough data for the ethnicity subgroup analysis of the association of MTHFR A1298C or MS A2756G polymorphism with the risk to cervical cancer.

With regard to CIN II/III, 8 studies were retrieved and 8 were included in the present meta-analysis according to the selection criteria. One study (8) was replaced with its updated version due to overlapping inclusion of study subjects in the most recent study. The eligible studies comprised 7 studies (3,6,17,21,24,26,27) with 1,936 cases and 2,858 controls for MTHFR C677T polymorphism (Table I) and the genotype distribution in the controls of all these studies was consistent with HWE. There were not enough studies available for the meta-analysis of the contribution of MTHFR A1298C or MS A2756G polymorphisms to susceptibility to CIN II/III.

DNA was prepared from blood samples or tissue for genotyping in all the studies. SNaPshot genotyping assay was adopted in 2 studies (3,21) and a TaqMan single nucleotide polymorphism (SNP) genotyping assay was used in 2 studies (6,17) with polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) employed in all

Table I. Characteristics of eligible studies included in this meta-analysis.

Study	Year of publication	Ethnicity	Mean age (Case/control)	Genotyping method	Source of controls	Ref.
Goodman *et al*	2001	Mixed	NS/NS	PCR-RFLP	Hospital-based, case-control study	(13)
Lambropoulos *et al*	2003	Caucasian	33.2/33.2	PCR-RFLP	Hospital-based, case-control study	(24)
Sull *et al*	2004	Asian	50.3/46.2	SNaPshot	Population-based, case-control study	(21)
Kang *et al*	2005	Asian	NS/NS	PCR-RFLP	Hospital-based, case-control study	(15)
Zoodsma *et al*	2005	Caucasian	NS/NS	TaqMan SNP	Hospital-based, case-control study	(17)
Delgado-Enciso *et al*	2006	Mixed	46/44	PCR-RFLP	Hospital-based, case-control study	(25)
Wang *et al*	2006	Asian	52.53/50.56	PCR-RFLP	Hospital-based, case-control study	(27)
Piyathilake *et al*	2007	Mixed	21.5/23.0	PCR-RFLP	Hospital-based, case-control study	(26)
Shekari *et al*	2008	Asian	48.55/48.81	PCR-RFLP	Hospital-based, case-control study	(22)
Kohaar *et al*	2010	Asian	49.4/48.2	SNaPshot	Hospital-based, case-control study	(3)
Tong *et al*	2011	Asian	50.8/45.7	TaqMan SNP	Hospital-based, case-control study	(6)
Prasad and Wilkhoo	2011	Asian	NS/NS	PCR-RFLP	Population-based, case-control study	(12)
Mostowska *et al*	2011	Caucasian	54.6/53.3	PCR-RFLP	Hospital-based, case-control study	(23)

NS, not stated; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; SNP, single nucleotide polymorphism.

of the remaining studies (13,15,22-27) to validate genotype distribution (Table I).

Results of the meta-analysis. Genotype distributions, allele frequencies, summary ORs and 95% CI for various genetic contrasts investigating the association of MTHFR C677T and A1298C or MS A2756G polymorphisms with cervical cancer and CIN II/III are listed in Tables II-V.

With respect to the MTHFR C677T polymorphism, no association was found with cervical cancer when a random effects model was adopted to conduct a worldwide allele comparison (T vs. C: P=0.53, OR=0.94, 95% CI=0.78-1.14, P=0.007 for heterogeneity; Table V). In the ethnicity subgroup analysis, an enhanced risk was demonstrated in Asian women (TT vs. CC: P=0.01, OR=1.41, 95% CI=1.07-1.86, P=0.05 for heterogeneity; Table V and Fig. 1A). By contrast, an inverse association was observed in Caucasian women (TT vs. CC: P=0.02, OR=0.65, 95% CI=0.45-0.93, P=0.99 for heterogeneity; Table V and Fig. 1B). Furthermore, meta-analyses of the contrasts in a recessive genetic model revealed that the 677T allele is more likely to reduce the risk of Caucasian women (TT+CT vs. CC: P=0.0005, OR=0.7, 95% CI=0.58-0.86, P=0.66 for heterogeneity; Table V and Fig. 1C), while the 677T allele is more likely to increase the risk of Asian women (TT vs. CC+CT: P=0.008, OR=1.38, 95% CI=1.08-1.75, P=0.12 for heterogeneity; Table V and Fig. 1D). No significant effect of MTHFR A1298C polymorphism on the susceptibility was found in worldwide populations (C vs. A: P=0.94, OR=0.99, 95% CI=0.82-1.20, P=0.83 for heterogeneity) and in Asian females (C vs. A: P=0.59, OR=1.06, 95% CI=0.85-1.33, P=0.96 for heterogeneity; Table V). Similarly, no association between MS A2756G polymorphism and cervical cancer was detected in the worldwide population (G vs. A: P=0.002, OR=0.65, 95% CI=0.21-1.98, P=0.0001 for heterogeneity; Table V).

With respect to association with CIN II/III, meta-analyses did not provide evidence to support an association between C677T polymorphism and susceptibility to CIN II/III in the worldwide population (T vs. C: P=0.86, OR=1.01, 95% CI=0.88-1.17, P=0.8 for heterogeneity; Table V) and in Asian women (T vs. C: P=0.49, OR=1.07, 95% CI=0.88-1.31, P=0.86 for heterogeneity; Table V).

Sensitivity analysis. Sensitivity analysis was conducted by sequentially omitting each study in turn under homozygote and recessive contrasts performed on a worldwide population and on ethnically defined subgroups to evaluate the robustness and

Table II. Distribution of MTHFR C677T genotypes and their allelic frequency associated with the risk of cervical cancer or CIN II/III.

Study		Genotype distribution, n				Chi-square	HWE P-value (control)	Ref.
		Total	CC	CT	TT			
Cervical cancer								
Lambropoulos *et al*	Case	21	11	8	2	0.699	0.403	(24)
	Control	91	42	37	12			
Sull *et al*	Case	246	73	115	58	0	0.990	(21)
	Control	454	153	221	80			
Kang *et al*	Case	79	27	32	20	0.482	0.487	(15)
	Control	74	30	32	12			
Zoodsma *et al*	Case	636	357	230	49	0.263	0.608	(17)
	Control	592	273	262	57			
Wang *et al*	Case	111	20	53	38	1.137	0.286	(27)
	Control	111	33	60	18			
Delgado-Enciso *et al*	Case	70	18	34	18	0.910	0.340	(25)
	Control	89	20	49	20			
Shekari *et al*	Case	200	170	28	2	0.372	0.542	(22)
	Control	200	125	68	7			
Kohaar *et al*	Case	164	113	47	4	0.277	0.599	(3)
	Control	231	161	65	5			
Tong *et al*	Case	146	53	65	28	0.792	0.373	(6)
	Control	427	152	198	77			
Prasad and Wilkhoo	Case	62	0	5	57	3.472	0.062	(12)
	Control	125	1	8	116			
Mostowska *et al*	Case	124	56	59	9	0.649	0.420	(23)
	Control	168	69	81	18			
CIN II/III								
Goodman *et al*	Case	150	73	67	10	0.656	0.418	(13)
	Control	179	93	75	11			
Lambropoulos *et al*	Case	64	27	29	8	0.622	0.430	(24)
	Control	91	42	37	12			
Sull *et al*	Case	176	50	90	36	0	0.990	(21)
	Control	454	153	221	80			
Zoodsma *et al*	Case	264	121	120	23	0.340	0.556	(17)
	Control	592	273	262	57			
Piyathilake *et al*	Case	80	59	16	5	0.034	0.853	(26)
	Control	355	223	116	16			
Kohaar *et al*	Case	39	28	11	0	0.277	0.599	(3)
	Control	231	161	65	5			
Tong *et al*	Case	160	54	74	32	0.792	0.373	(6)
	Control	427	152	198	77			

CIN, cervical intraepithelial neoplasia; HWE, Hardy-Weinberg equilibrium; MTHFR, methylenetetrahydrofolate reductase.

plausibility of the meta-analysis. The pooled ORs (including 95% CI) from various contrasts were not significantly altered (data not shown), indicating that the summary estimate of the effect of MTHFR C677T, MTHFR A1298C and MS A2756G polymorphisms on the risk of cervical cancer and CIN II/III was not altered during the sensitivity analysis.

Publication bias. The Begg's funnel plot and Egger's test were conducted to estimate the publication bias of the included studies. The funnel plot for the comparison of the 677C allele with the 677T allele provided limited evidence on obvious asymmetry. No publication bias by Egger's test was detected for the comparison in homozygote (TT vs. CC), dominant (TT+CT vs. CC) and recessive models (TT vs. CT+CC) for both cervical cancer and CIN II/III (Table VI). Similarly, there was no statistical evidence suggesting publication bias for the comparison of the three models of MTHFR A1298C polymorphism for cervical cancer (Table VI). Furthermore, no

Table III. Distribution of MTHFR A1298C genotypes and their allelic frequency associated with the risk of cervical cancer.

Study		Genotype distribution, n				Chi-square	HWE P-value (control)	Ref.
		Total	CC	CT	TT			
Kang *et al*	Case	79	55	22	2	0.895	0.344	(15)
	Control	84	58	25	1			
Delgado-Enciso *et al*	Case	70	2	24	44	0.002	0.969	(25)
	Control	89	2	23	64			
Kohaar *et al*	Case	164	58	83	23	2.293	0.123	(3)
	Control	231	85	119	27			
Tong *et al*	Case	148	89	57	2	0.215	0.643	(6)
	Control	428	278	132	18			
Mostowska *et al*	Case	124	56	59	9	0.649	0.420	(23)
	Control	168	69	81	18			

HWE, Hardy-Weinberg equilibrium; MTHFR, methylenetetrahydrofolate reductase.

Table IV. Distribution of MS A2756G genotypes and their allelic frequency associated with the risk of cervical cancer.

Study		Genotype distribution, n				Chi-square	HWE P-value (control)	Ref.
		Total	CC	CT	TT			
Kang *et al*	Case	65	53	10	2	0.835	0.361	(15)
	Control	72	58	14	0			
Shekari *et al*	Case	200	181	14	5	1.834	0.176	(22)
	Control	200	118	63	14			
Mostowska *et al*	Case	124	72	44	8	1.900	0.168	(23)
	Control	168	109	49	10			

HWE, Hardy-Weinberg equilibrium.

publication bias was observed for the G vs. A allele contrast of MS A2756G polymorphism for cervical cancer (t=0.61, P=0.654).

Discussion

Cervical cancer is one of the three major malignancies found in female cancer patients worldwide, accounting for 250,000 deaths/year, with higher incidences being observed in developing countries compared with developed countries (17,26,28). Therefore, it would be useful to have precise susceptibility information on cervical carcinogenesis to develop effective, specific and individualized disease prevention programs for different populations (37,38).

Infection with oncogenic subtypes of HPV has been confirmed to play a crucial etiological role in the development of cervical cancer (32,38,39). However, infection with high-risk HPV alone is not sufficient to cause cervical neoplasia. An increasing number of studies suggest that oral contraceptives, smoking, host genetic factors and epigenetic changes enhance susceptibility to the development of cervical intraepithelial neoplasia and invasive cancer (23,30). Recently, epidemiological studies have reported that heritable factors, including genetic polymorphisms, contributed to ~64% of the familial risks for cervical cancer (23,30).

Variation of several candidate genes involved in the one-carbon metabolism pathway may explain some of the individual differences in cervical tumorigenesis, including MTR, BHMT, MTHFR, MTHFD1 and MS, among which MTHFR and MS are two of the most commonly investigated candidate genes.

MTHFR catalyzes the conversion of 5,10-methylenetetrahydrofolate (5,10-methylene-THF) to 5-methyltetrahydrofolate (5-methyl-THF). 5,10-methylene-THF is a substrate necessary for thymidine synthesis, while 5-methyl-THF acts as a substrate for the remethylation of homocysteine under the catalysis of MS, resulting in methionine synthesis, which plays a role as a substrate for S-adenosyl methionine (SAM) synthesis. SAM is a universal methyl donor necessary for DNA and protein methylation. Increased MTHFR function results in low levels of 5,10-methylene-THF, thereby leading to the misincorporation of dUTP into DNA, which in turn causes double strand breaks (9,31). Conversely, decreased MTHFR activity may result in low levels of 5-methyl-THF and, thus, be responsible for DNA hypomethylation. These are common features in cancer development (9,21,23,24).

Table V. Meta-analysis of various genetic comparisons investigating the association of MTHFR C677T, MTHFR A1298C and MS A2756G polymorphisms with cervical cancer or CIN II/III susceptibility.

Analysis model	Ethnicity	Random effects model OR (95% CI)	Fixed effects model OR (95% CI)	P-value for heterogeneity	P-value for fixed effects model
MTHFR C677T in cervical cancer					
T vs. C	Asian	0.98 (0.73-1.32)	1.01 (0.87-1.17)	0.002	0.93
	Caucasian	0.82 (0.69-0.97)	0.82 (0.69-0.97)	0.87	0.02
	Total	0.94 (0.78-1.14)	0.93 (0.83-1.103)	0.007	0.17
TT vs. CC	Asian	1.4 (0.87-2.26)	1.41 (1.07-1.86)	0.05	0.01
	Caucasian	0.65 (0.45-0.93)	0.65 (0.45-0.93)	0.99	0.02
	Total	1.07 (0.73-1.58)	1.06 (0.86-1.31)	0.008	0.6
TT+CT vs. CC	Asian	0.98 (0.62-1.54)	0.94 (0.79-1.13)	<0.0001	0.52
	Caucasian	0.7 (0.58-0.86)	0.7 (0.58-0.86)	0.66	0.0005
	Total	0.89 (0.66-1.18)	0.83 (0.73-0.94)	<0.0001	0.004
TT vs. CC+CT	Asian	1.36 (0.95-1.95)	1.38 (1.08-1.75)	0.12	0.008
	Caucasian	0.75 (0.53-1.07)	0.75 (0.53-1.07)	0.92	0.11
	Total	1.13 (0.84-1.52)	1.14 (0.94-1.38)	0.05	0.18
MTHFR C677T in CIN II/III					
T vs. C	Total	1.01 (0.88-1.17)	1.01 (0.88-1.17)	0.8	0.86
	Asian	1.00 (0.67-1.48)	1.07 (0.88-1.31)	0.86	0.49
TT vs. CC	Total	1.13 (0.86-1.49)	1.12 (0.86-1.48)	0.91	0.4
	Asian	1.25 (0.88-1.80)	1.25 (0.87-1.78)	0.76	0.22
TT+CT vs. CC	Total	1.02 (0.85-1.23)	1.02 (0.86-1.22)	0.37	0.79
	Asian	1.14 (0.89-1.48)	1.14 (0.89-1.47)	0.67	0.3
TT vs. CC+CT	Total	1.09 (0.85-1.39)	1.08 (0.84-1.39)	0.93	0.54
	Asian	1.16 (0.85-1.59)	1.16 (0.84-1.58)	0.85	0.37
MTHFR A1298C in cervical cancer					
C vs. A	Total	0.99 (0.82-1.20)	0.99 (0.82-1.20)	0.83	0.94
	Asian	1.06 (0.85-1.33)	1.06 (0.85-1.33)	0.96	0.59
CC vs. AA	Total	0.89 (0.55-1.41)	0.85 (0.54-1.34)	0.43	0.48
	Asian	0.96 (0.40-2.27)	0.99 (0.57-1.71)	0.25	0.96
CC+AC vs. AA	Total	1.05 (0.83-1.31)	1.04 (0.83-1.31)	0.81	0.7
	Asian	1.12 (0.86-1.45)	1.12 (0.86-1.45)	0.8	0.39
CC vs. AA+AC	Total	0.81 (0.52-1.24)	0.80 (0.56-1.16)	0.31	0.24
	Asian	0.91 (0.35-2.38)	0.98 (0.58-1.63)	0.19	0.93
MS A2756G in cervical cancer					
G vs. A	Total	0.65 (0.21-1.98)	0.62 (0.46-0.84)	0.0001	0.002
GG vs. AA	Total	0.79 (0.17-3.62)	0.64 (0.33-1.21)	0.03	0.17
GG+AG vs. AA	Total	0.63 (0.26-1.50)	0.61 (0.47-0.79)	0.0001	0.002
GG vs. AA+AG	Total	0.81 (0.25-2.62)	0.72 (0.38-1.38)	0.11	0.32

CIN, cervical intraepithelial neoplasia; OR, odds ratio; CI, confidence interval; MTHFR, methylenetetrahydrofolate reductase.

The gene for MTHFR is located on chromosome 1p36 with 11 exons (44) and SNPs within the coding region are associated with DNA hypomethylation, which constitutes a hallmark of human cancer cells (1,24,25). Two common mutations in the MTHFR gene are C677T and A1298C. MTHFR C677T polymorphism leads to substitution of alanine by valine at the amino acid position 222, which affects the catalytic domain of the enzyme and decreases its affinity for its cofactor (9). This altered form of enzyme results in a thermolabile protein and is associated with reduced enzymatic activity (9,10). Thus, elevated homocysteine levels may be attributed to a correspondingly decreased activity of this enzyme in individuals homozygous and heterozygous for the variation (9), which results in abnormalities in DNA methylation (23). MTHFR

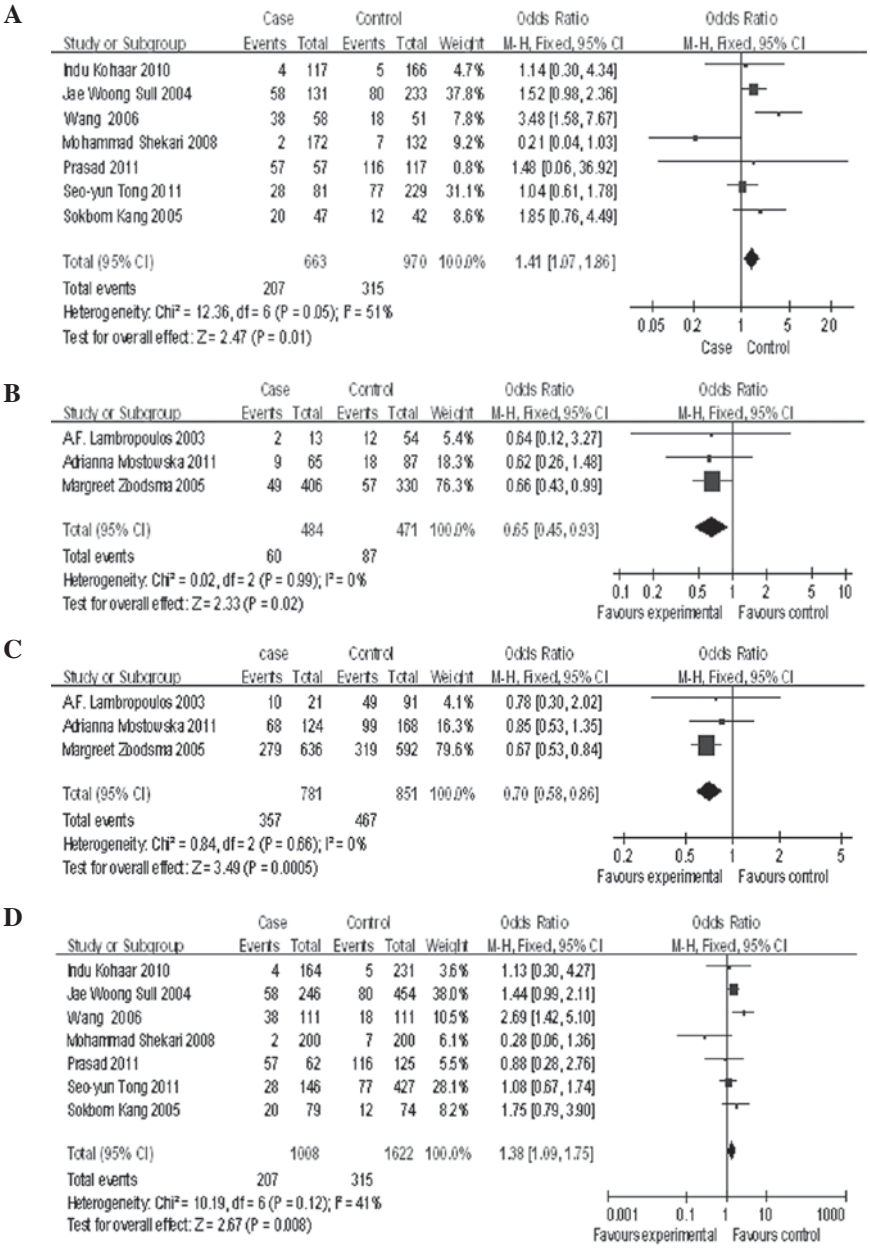

Figure 1. Meta-analysis for the association between MTHFR C677T polymorphism and cervical cancer susceptibility stratified by ethnicity. Point estimates of the OR together with 95% CI values for each study obtained with a fixed effects model are plotted. (A) Analysis of cervical cancer risk for TT genotype when compared with the CC genotype in the Asian population. (B) Analysis of cervical cancer risk for TT genotype when compared with the CC genotype in the Caucasian population. (C) Analysis of the comparison in TT + CT vs. CC in the Caucasian population. (D) Analysis of the comparison in TT vs. CC+CT in the Asian population. OR, odds ratio; CI, confidence interval; MTHFR, methylenetetrahydrofolate reductase.

A1298C polymorphism converts a glutamine to alanine at the amino acid position 429 (9), which is located within the regulatory domain of this protein (9). However, this alteration does not appear to affect the function of MTHFR by itself, but may reduce MTHFR activity when it is heterozygous with the 1556 G→A, 1743 G→A and 1958 C→T polymorphisms (9,34,35).

Due to both MS and MTHFR functioning in the same metabolic pathway sequentially, the affect of MS gene polymorphism constitutes an additional interesting research issue. MS catalyzes the methyl transfer from homocysteine to methionine with cobalamine as a co-factor to maintain adequate intracellular SAM levels for DNA methylation, which is believed to suppress cancer development. The common variant in MS is an A-to-G transversion at position 2756 (MS A2756G), which leads to the replacement of aspartate by glycine, resulting in altered enzyme activity and, thus, affecting DNA methylation (1,35).

There is an increasing number of studies demonstrating that MTHFR and MS polymorphisms play different roles in influencing susceptibility to breast, colorectal, pancreatic, hepatocellular and prostate cancers. Particularly, the effect of MTHFR and MS polymorphisms on the risk of CIN II/III and cervical cancer also remains inconsistent (12,15-20). The MTHFR C677T gene variant has been associated with a risk of cervical carcinogenesis in certain cohorts (13,17,21,23), while this MTHFR gene variant has been associated with protection against CIN II/III or cervical cancer (15), or has not been confirmed as a risk factor for this type of cancer in

Table VI. Publication bias detection for MTHFR C677T and MTHFR A1298C polymorphisms.

Genetic type	Coefficient	SE	t-value	P-value	95% CI of intercept
C677T for cervical cancer					
TT vs. CC	5.480	4.639	1.18	0.268	(-5.015 to 15.976)
TT+CT vs. CC	-1.352	2.836	-0.48	0.645	(-7.766 to 5.063)
TT vs. CT+CC	0.948	1.621	0.58	0.573	(-2.720 to 4.616)
C677T for CIN II/III					
TT vs. CC	-1.978	2.113	-0.94	0.418	(-8.701 to 4.746)
TT+CT vs. CC	13.775	8.494	1.62	0.180	(-9.807 to 37.358)
TT vs. CT+CC	0.427	1.846	0.23	0.832	(-5.448 to 6.302)
A1298C for cervical cancer					
CC vs. AA	-0.867	1.051	-0.92	0.385	(-3.393 to 1.459)
CC+CA vs. AA	6.484	4.034	1.61	0.206	(-6.355 to 19.323)
CC vs. CA+AA	-2.077	1.541	-1.35	0.271	(-6.980 to 2.828)

CI, confidence interval; CIN, cervical intraepithelial neoplasia; MTHFR, methylenetetrahydrofolate reductase.

other populations (3,9,11,23,24,35). Similarly, the influence of MS A2756G polymorphism on the susceptibility of cervical tumorigenesis also remains controversial (11,15,23). Therefore, a systematic meta-analysis with regard to the three most investigated polymorphisms of MTHFR (C677T and A1298C) and MS (A2756G) genes is needed in order to determine the influence of MTHFR or MS polymorphisms on susceptibility to CIN II/III or cervical cancer.

A total of 13 studies with 1,936 cases and 2,858 controls were identified for the investigation of MTHFR C677T and A1298C polymorphisms, while 3 studies with 389 cases and 440 controls were eligible for the investigation of MS A2756G polymorphism in the present study. No significant associations were found in the worldwide population between polymorphisms of the MTHFR gene (C677T and A1298C) or the MS gene (A2756G), and CIN II/III or cervical cancer. However, stratified analysis by ethnicity demonstrated that increased susceptibility was restricted to Asian females when homozygous and recessive genetic model contrasts were conducted. By contrast, an inverse association of the MTHFR C677T polymorphism and cervical cancer was observed in Caucasian females. It has been established that one-carbon metabolism may be affected not only by genes encoding enzymes involved in this pathway, but also by dietary factors such as folate, vitamin B and alcohol intake (36). A previous study demonstrated that the MTHFR TT genotype constitutes a protective factor against susceptibility to colorectal cancer in folate-replete subjects, while this genotype conferred an enhanced risk of colorectal cancer in combination with a folate-deficient status (37). Therefore, the dipartite results of the effects of MTHFR C677T polymorphism between Asian and Caucasian females in the present study may be due to the higher folate intake in North America and Europe in dietary supplement use and food fortification (38). This may also be due to ethnic differences in additional genetic factors and different environments. No associations were found between MTHFR A1298C or MS A2756G gene polymorphisms and cervical cancer in the different ethnic groups partially due to the fact that each of these single gene variations do not appear to affect the function of MTHFR or MS by itself until it is heterozygous with other gene polymorphisms on the same sequence.

The sensitivity analysis in the present study indicated that the summary estimate of effect of MTHFR C677T, MTHFR A1298C and MS A2756G polymorphisms on the risk of cervical cancer and CIN II/III was robust, and was not significantly altered following various comparisons. Moreover, the evaluation of publication bias in the present study did not indicate the existence of such bias towards the observed association between MTHFR C677T or A1298C variant and the risk for CIN II/III or cervical cancer in the comparison of variant alleles, suggesting that the results were credible and stable.

However, there are several limitations that may limit the strength of the conclusions. Firstly, the statistical power of the meta-analysis is relatively low, due to the fact that the number of cases of many of the included studies was relatively small and the fact that the controls were not defined uniformly and were not representative enough (1,13,24). Secondly, different racial distributions and genetic heterogeneity existed among the studied populations, which resulted in conflicting results and led to the inability to examine the potential susceptibility of MTHFR or MS polymorphisms (23,39). Thirdly, the present study focused only on the three most investigated SNPs associated with CIN II/III or cervical cancer due to the limited number of informative studies. Fourthly, most data were not stratified according to the investigated SNPs by behavioral and environmental cofactors, such as dietary folate intake, folate and other micronutrient status within the body, HPV infection, hormone or oral contraceptives, smoking, which might make it difficult to investigate the joint effects among pairs of variables modifying the susceptibility of cervical cancer or precancerous conditions. Moreover, variations in laboratory procedures, such as methods of data collection and genotyping, could also explain the inconsistent results. Therefore, a more precise analysis needs to be performed upon availability of data from additional investigations with an improved design.

In conclusion, the present meta-analysis supports the hypothesis that the MTHFR 677TT polymorphism is associ-

ated with an increased risk of cervical cancer in Asian females, while an inverse association applies to Caucasian females. Meanwhile, no association was detected between the MTHFR C677T polymorphism and susceptibility to CIN II/III overall or in ethnically defined populations. Similarly, MTHFR A1298C and MS A2756G polymorphisms did not appear to be associated with overall cervical cancer risk or in ethnically defined populations. Larger population-based surveys concerning gene-gene, gene-nutrients and gene-behavioral risk factors interactions in a specific population are needed to further determine the role of MTHFR and MS gene polymorphisms in the risk of cervical cancer.

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