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Association of methylenetetrahydrofolate reductase C677T and A1298C polymorphisms with colorectal cancer risk: A meta-analysis

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Abstract. Colorectal cancer (CRC) is one of the most common types of cancer worldwide and a leading cause of cancer-related mortality. This meta-analysis was conducted to determine the effect of methylenetetrahydrofolate reductase (MTHFR) mutants on the risk of CRC. A literature search was conducted on PubMed, Medline and the China National Knowledge Infrastructure (CNKI) databases. Eligible studies were collected based on rigorous criteria of inclusion. The pooled odds ratios (ORs) with 95% confidence intervals (CIs) were calculated by the fixed- or random-effects model. After all the studies were pooled, the OR of CRC for individuals carrying the MTHFR 677TT genotype, compared to the CC genotype, was 0.89 (95% CI: 0.82-0.97). When analyzed by ethnicity, Asians with the MTHFR 1298CC genotype exhibited a decreased risk of CRC (OR=0.69; 95% CI: 0.54-0.89). In a mixed population, a significantly reduced risk of CRC was observed among carriers of the 677TT (OR=0.86; 95% CI: 0.76-0.96) and the 1298CC (OR=0.82; 95% CI: 0.69-0.98) genotypes, compared to the wild-type homozygous genotype. In the subgroup of colon cancer, the OR of 677TT vs. CC+CT was 0.83 (95% CI: 0.72-0.96) and the OR of 1298CC vs. AA+AC was 0.81 (95% CI: 0.69-0.96). In the rectal cancer subgroup, the OR of 677TT vs. CC+CT was 0.86 (95% CI: 0.77-0.97). Therefore, this meta-analysis suggested that the MTHFR 677T and 1298C alleles were associated with a low risk of CRC.

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Introduction

Colorectal cancer (CRC) is one of the most common types of cancer worldwide (1). It is the fourth leading cause of cancer-related mortality among males and the third among females. Furthermore, the mortality rate of CRC in developed countries was shown to be higher compared to that in developing countries (3).

Folate is a type of water-soluble B vitamin and an essential nutrient required for human metabolism. Folate plays a key role in the formation of S-adenosylmethionine, which is the universal methyl donor for DNA methylation, as well as in the formation of purine and thymidine for DNA synthesis (4). Folate deficiency increases the risk of tumorigenesis through one of the following mechanisms: by leading to aberrant DNA methylation, which may in turn lead to an altered expression of critical tumor suppressor genes and proto-oncogenes; or by causing imbalances in the pools of nucleotide precursors, leading to DNA strand breaks and mutations and disruption of DNA integrity and repair (4,5).

The methylenetetrahydrofolate reductase (MTHFR) is an enzyme that is crucial in the metabolism of folate (6). MTHFR catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the primary methyl donor for the remethylation of homocysteine to methionine. The gene encoding MTHFR is located on chromosome 1p36.3 (7). Two common single-nucleotide polymorphisms of MTHFR are MTHFR C677T (Ala222Val, rs1801133) and A1298C (Glu429Ala, rs1801131). The C677T variant enhances enzyme thermolability and is associated with decreased activity of the MTHFR enzyme (8). The A1298C variant (Glu429Ala, rs1801131) is a missense mutation leading to reduced MTHFR enzyme activity (9,10). The homozygous genotypes of MTHFR C677T and A1298C are associated with higher homocysteine levels, which may lead to DNA hypomethylation and increased cancer prevalence. However, the decreasing enzyme activity results in higher 5,10-methylenetetrahydrofolate and thymidine levels and, thus, increased DNA synthesis and repair. Therefore, MTHFR polymorphisms are regarded as a protective factor against tumor development (8,11).

It was first reported by Chen *et al* (10) that the homozygote of the MTHFR 677T allele was associated with a reduced CRC risk. Previous studies reported that the MTHFR 677T or 1298C allele exerted a protective effect compared to the wild-type genotypes (12,13). However, other studies reported opposing results, i.e., that mutant genotypes were associated with an increased risk of developing CRC (14,15).

A meta-analysis of all published studies was conducted to determine the effect of MTHFR mutants on CRC risk. In a subgroup analysis, the study subjects were classified by ethnicity and tumor location to provide comprehensive evidence on the association of MTHFR C677T and A1298C with CRC.

Materials and methods

Study identification and selection. A literature search was conducted on PubMed, Medline and China National Knowledge Infrastructure (January, 1991-September, 2012) databases, using the following keywords and subject terms: 'MTHFR', 'polymorphism' and 'colon cancer' or 'rectal cancer'. All the studies in our meta-analysis were required to meet the following inclusion criteria: i) case-control studies; ii) raw data to calculate odds ratios (ORs) with 95% confidence intervals (95% CIs); iii) in case of the same results published in multiple studies, the most recent publication or the largest sample was considered. A given study was excluded from this meta-analysis when: i) the genotype or allele frequencies were not reported, ii) the study design was not case-control, iii) the association between MTHFR polymorphisms and colorectal adenoma was investigated.

Data extraction. Data were carefully and independently collected according to the genotypes MTHFR C677T or A1298C. Two authors extracted the following information from the eligible studies: first author's name, publication year, country, ethnicity of participants and number of cases and controls. In our study, ethnicities were classified as European and American, Asian, African and mixed.

Statistical analysis. The association between MTHFR C677T and A1298C gene polymorphisms and CRC risk was assessed by using the codominant (677CT vs. CC; 677TT vs. CC; 1298AC vs. AA; 1298CC vs. AA), the dominant (677CT+TT vs. CC; 1298AC+CC vs. AA) and the recessive (677TT vs. CC+CT; 1298CC vs. AA+AC) models. The same procedures were applied for the MTHFR A1298C genotype. Subgroup analyses were performed by tumor location and ethnicity of the control groups.

The strength of association of the MTHFR gene polymorphisms with CRC was measured by the ORs (1) together with the 95% CIs. The significance of the pooled ORs was determined by the Z-test and $P<0.05$ was considered to indicate a statistically significant difference. The Chi-square test was first used to assess whether the distribution of genotypes among controls conformed to the Hardy-Weinberg equilibrium (HWE), with $P<0.05$ considered a departure from HWE. The Q-test was used to assess heterogeneity among the studies. When $P<0.05$, the heterogeneity was considered to indicate a statistically significant difference. The I^2 index was used to quantify the percentage of the total variation among studies when heterogeneity was calculated. The I^2 value ranged from 0 to 100%, with 25, 50 and 75% expressing low, moderate and high heterogeneity, respectively. When $I^2<50\%$, a fixed-effects model (the Mantel-Haenszel method) was applied to estimate the pooled results. Otherwise, the random-effects model (the DerSimonian-Laird method) was used.

Publication bias was visually investigated in a funnel plot of log (OR) against its standard error (SE). An asymmetric plot suggested possible publication bias. The degree of asymmetry was assessed via the Egger's test ($P<0.05$ was considered publication bias). A sensitivity analysis was performed by omitting each study in turn to assess the stability of the results.

All the analyses were performed with Stata software version 11.0 (StataCorp, College Station, TX, USA). All the P-values were two-sided.

Results

Selection of studies. A total of 302 studies were identified during the literature search and 232 were excluded due to departures from the inclusion criteria. Eventually, 70 studies were included (1,2,6,10,12-77). One study, conducted by Lee *et al* (41), consisted of three individual case-control studies and was handled as three populations; furthermore, the studies by Keku *et al* (12) and Lima *et al* (28), included two populations each. This meta-analysis included a larger population compared to previous meta-analyses. Of the 74 case-control studies included in the 70 publications, 74 studies investigated MTHFR C677T (29,783 cases and 41,772 controls) and 39 investigated MTHFR A1298C (13,285 cases and 20,164 controls). In total, 33 populations were from Europe and America; 31 were from Asia; 4 were from Africa and the remaining were mixed populations. In addition, a subanalysis was conducted by tumor location. Twenty-one of the 74 studies provided detailed data on colon and 14 on rectal cancer. The characteristics of the studies are listed in Table I.

Meta-analysis. The results of the meta-analysis are presented in Table II. There was obvious heterogeneity for all the models, except for the MTHFR A1298C additive model (CA vs. AA, $P=0.307$, $I^2=9.2\%$) and the random-effects model was used to estimate the pooled data. The meta-analysis of the 74 populations demonstrated that MTHFR C677T was associated with reduced CRC risk under the homozygote (TT vs. CC: OR=0.89; 95% CI: 0.82-0.97; P=0.009) (Fig. 1) and recessive (TT vs. CC+CT: OR=0.89; 95% CI: 0.82-0.96; P=0.003) models (Fig. 2). However, the MTHFR 1298C allele was not associated with a significantly decreased risk of CRC.

The association of MTHFR C677T and A1298C polymorphisms with CRC was further stratified by ethnicity. As shown in Table II, no significant association was observed between MTHFR C677T and the risk of CRC under any genetic models, in any of the populations. However, Asians carrying the MTHFR 1298CC genotype exhibited a reduced risk of CRC. The OR of CC vs. AA was 0.69 (95% CI: 0.54-0.89) and the OR of CC vs. AA+AC was 0.69 (95% CI: 0.54-0.88). In the mixed populations, the pooled analysis demonstrated that carriers of MTHFR 677TT and 1298CC were more common among CRC patients than among controls when compared to individuals with wild-type genotypes (677TT vs. CC: OR=0.86;

Table I. Characteristics of the studies included in the meta-analysis.

First author	Year	Country	Location	MTHFR 677C/T (case/control)				MTHFR 1298A/C (case/control)				Refs.
				CC	CT	TT	P-value	AA	AC	CC	P-value	
Europe/America												
Chen *et al*	1996	USA	NR	67/280	64/263	13/84	0.08					10
Ma *et al*	1997	USA	NR	92/145	92/132	18/49	0.04					16
Ryan *et al*	2001	Ireland	NR	49/439	73/326	14/83	0.05					17
Keku *et al*	2002	USA	Colon	144/265	140/223	24/51	0.68	156/237	132/236	21/68	0.44	12
Shannon *et al*	2002	Australia	Colon	249/533	197/560	55/114	0.05					14
Sachse *et al*	2002	UK	NR	238/271	199/272	53/49	0.09					18
Heijmans *et al*	2003	Netherlands	NR	7/399	7/329	4/65	0.81					13
Pufulete *et al*	2003	UK	NR	36/41	19/29	8/6	0.78	34/47	22/26	7/3	0.80	19
Plaschke *et al*	2003	Germany	NR	133/149	120/159	34/38	0.65	134/154	124/151	29/41	0.67	20
Toffoli *et al*	2003	Italy	Colon	93/83	145/140	38/56	0.83	122/133	129/121	25/25	0.74	21
Ulvik *et al*	2004	Norway	NR	1,103/1,092	899/886	157/212	0.10					22
Landi *et al*	2005	Spain	NR	128/109	158/139	64/61	0.17	189/170	146/127	25/22	0.79	23
Koushik *et al*	2006	USA	NR	166/355	145/327	38/112	0.01	154/389	166/332	33/85	0.26	24
Van Guelpen *et al*	2006	Sweden	NR	123/212	85/161	12/42	0.17	85/189	103/173	32/50	0.29	25
Battistelli *et al*	2006	Italy	NR	32/30	40/51	21/19	0.75					26
Osian *et al*	2007	Romania	NR	38/47	25/17	6/3	0.38	33/41	32/25	4/1	0.19	27
Lima *et al*	2007	Brazil	NR	36/143	46/127	15/30	0.82	68/191	28/93	6/16	0.30	28
Theodoratou *et al*	2008	Scotland	NR	447/439	441/455	111/116	0.91	465/462	425/445	106/102	0.73	29
Sharp *et al*	2008	Scotland	NR	117/170	111/177	23/47	0.93	105/177	111/157	29/60	0.01	30
Eklöf *et al*	2008	Sweden	NR	123/212	85/160	12/42	0.15					31
Küry *et al*	2008	France	NR	435/457	452/515	136/149	0.84	484/577	432/443	107/101	0.23	32
Derwinger *et al*	2009	Sweden	NR	273/167	216/107	55/25	0.19					33
de Vogel *et al*	2009	Netherlands	NR	318/876	320/750	51/167	0.72	299/735	275/774	110/258	0.02	34
Gallegos-Arreola *et al*	2009	Mexico	NR	124/59	126/79	119/32	0.54					36
Fernández-Peralta *et al*	2010	Spain	NR	89/44	52/50	2/9	0.32	84/57	53/44	6/2	0.05	35
Komlosi *et al*	2010	Hungary	Colon	208/216	196/186	68/59	0.01					37
			Rectum	190/226	231/194	58/58						
Karpinski *et al*	2010	Poland	NR	74/71	97/55	15/14	0.49					38
Eussen *et al*	2010	Europe	NR	567/1,019	608/1,076	154/271	0.61	605/1,099	574/1,007	151/259	0.22	40
Pardini *et al*	2011	Czech	NR	317/613	307/627	42/136	0.18	281/583	309/638	76/156	0.35	1
Vossen *et al*	2011	Germany	Colon	454/795	502/807	122/209	0.85					39
			Rectum	283/795	321/807	80/209						
Lee *et al*	2012	USA/NHS	NR	89/165	66/140	20/48	0.04	72/181	82/136	21/38	0.11	41
Lee *et al*	2012	USA/HPFS	NR	72/140	69/127	17/51	0.02	73/147	73/133	7/32	0.81	41
Lee *et al*	2012	USA/PHS	NR	89/159	94/124	15/50	0.00	101/167	100/154	12/44	0.36	41
Asia												
Park *et al*	1999	Korea	NR	65/140	107/246	28/74	0.05					42
Matsuo *et al*	2002	Japan	Colon	23/81	39/124	10/36	0.30	50/157	19/75	3/9	0.02	43
			Rectum	16/81	42/124	12/36		44/157	25/75	0/9		
Huang *et al*	2003	China	NR	36/40	40/33	6/9	0.58					44
Yin *et al*	2004	Japan	Colon	154/278	180/367	48/133	0.53	236/515	128/244	18/19	0.11	15
			Rectum	110/278	144/367	36/133		192/515	90/244	8/19		
Kim *et al*	2004	Korea	Colon	24/83	67/109	20/33	0.77					45
			Rectum	62/83	55/109	15/33						
Matsuo *et al*	2005	Japan	NR	106/289	114/348	36/134	0.10	163/479	85/257	9/31	0.63	46
Jiang *et al*	2005	China	Colon	19/134	31/143	3/62	0.03	36/226	17/103	0/6	0.03	47
			Rectum	32/134	28/143	12/62		57/226	13/103	1/6		
Miao *et al*	2005	China	NR	53/133	87/201	58/86	0.52					48
Otani *et al*	2005	Japan	NR	32/51	49/114	25/57	0.68	73/156	32/63	1/5	0.64	49
Wang *et al*	2006	India	Colon	53/255	6/36	0/0	0.26	32/105	22/135	5/51		50
			Rectum	204/255	37/36	2/0		109/105	108/135	26/51		
Chang *et al*	2007	China	NR	85/92	86/87	24/16	0.47	120/127	65/55	10/13	0.05	51
Zeybek *et al*	2007	Turkey	NR	18/64	27/65	7/15	0.80					52

Table I. Continued.

First author	Year	Country	Location	MTHFR 677C/T (case/control)				MTHFR 1298A/C (case/control)				Refs.
				CC	CT	TT	P-value	AA	AC	CC	P-value	
Asia												
Jin *et al*	2007	China	Colon	99/211	117/325	35/136	0.60					53
			Rectum	82/211	92/325	20/136						
Cao *et al*	2008	China	Colon	30/121	53/183	22/66	0.82	66/239	38/119	1/13	0.70	2
			Rectum	79/121	101/183	30/66		138/239	67/119	5/13		
Mokarram *et al*	2008	Iran	Colon	64/40	80/31	7/10	0.31					54
Zhang *et al*	2008	China	NR	97/91	136/139	67/69	0.26					56
Haghighi *et al*	2009	Iran	NR	117/94	68/80	49/83	0.00					55
Promthet *et al*	2010	Thailand	Colon	104/94	26/31	0/5	0.24	43/54	84/71	3/5	0.00	57
Cui *et al*	2010	Korea	NR	622/540	923/863	284/297	0.13					6
Naghibalhossaini *et al*	2010	Iran	Colon	64/150	80/68	7/13	0.16					58
Chandy *et al*	2010	India	NR	74/66	25/19	1/1	0.78	22/22	70/50	8/14	0.11	60
Zhu *et al*	2010	China	NR	88/50	102/53	26/8	0.23					61
Yang *et al*	2010	China	NR	58/62	61/75	22/28	0.52					75
Kim *et al*	2011	Korea	NR	30/15	30/21	7/17	0.13	44/36	22/16	1/1	0.61	59
Zhu *et al*	2011	China	NR	29/49	42/41	15/10	0.74					76
Kang *et al*	2011	Korea	NR	87/145	134/238	34/65	0.04					62
Prasad and Wilkhoo	2011	India	NR	97/228	12/12	1/1	0.07					63
Sameer *et al*	2011	India	Colon	23/121	7/27	6/12	0.00					64
			Rectum	36/121	11/27	3/12						
Li *et al*	2011	China	NR	68/55	54/64	15/26	0.33	88/76	47/60	2/9	0.53	65
Yin *et al*	2012	China	NR	124/139	167/178	79/53	0.74					66
Kim *et al*	2012	Korea	Colon	121/205	185/289	57/162	0.00					67
			Rectum	109/205	164/289	57/162						
Africa												
Keku *et al*	2002	USA	NR	198/264	43/59	3/6	0.21	157/217	78/99	8/13	0.69	12
Lima *et al*	2007	Brazil	NR	4/143	5/127	1/30	0.82	5/191	4/93	1/16	0.30	28
El Awady *et al*	2009	Egypt	NR	6/44	23/20	6/4	0.41	5/26	21/37	9/15	0.09	68
Guimarães *et al*	2011	Brazil	Colon	17/92	28/79	9/17	0.99	39/127	12/49	3/12	0.02	69
			Rectum	31/92	22/79	6/17		28/127	26/49	5/12		
Mixed												
Slattery *et al*	1999	USA	Colon	673/827	655/787	139/207	0.34					70
Curtin *et al*	2004	USA	Colon	734/887	724/858	150/227	0.37	757/929	698/827	153/216	0.12	71
Le Marchand *et al*	2005	USA	Colon	295/987	246/779	56/255	0.00					77
			Rectum	99/987	90/779	31/255						
Murtaugh *et al*	2007	USA	Rectum	357/466	301/392	84/112	0.04	360/436	317/424	65/110	0.65	72
Iacopetta *et al*	2009	Australia	NR	382/428	386/429	82/101	0.67					73
Reeves *et al*	2009	Australia	NR	105/101	83/91	18/19	0.82	92/86	89/98	25/27	0.91	74

NR, not reported; P-value is for Hardy-Weinberg equilibrium.

95% CI: 0.76-0.96; TT vs. CC+CT: OR=0.85; 95% CI: 0.76-0.95) (1298CC vs. AA: OR=0.82; 95% CI: 0.69-0.98; and CC vs. AA+AC: OR=0.83; 95% CI: 0.70-0.99).

Furthermore, the participants were stratified by tumor location. A significantly decreased risk of CRC was observed under the recessive model of MTHFR 677TT (OR=0.83; 95% CI: 0.72-0.96) and 1298CC (OR=0.81; 95% CI: 0.69-0.95) in the colon cancer group. In addition, the stratified analysis revealed that MTHFR C677T was associated with reduced risk of rectal cancer. The OR under the recessive model was 0.86 (95% CI: 0.77-0.97). The main results are presented in Table II.

Sensitivity analysis and publication bias. The elimination of each individual study imparted no qualitative difference on the pooled OR values, indicating that the final results of the meta-analysis were relatively stable. The publication bias of the studies was determined by a funnel plot and the Egger's

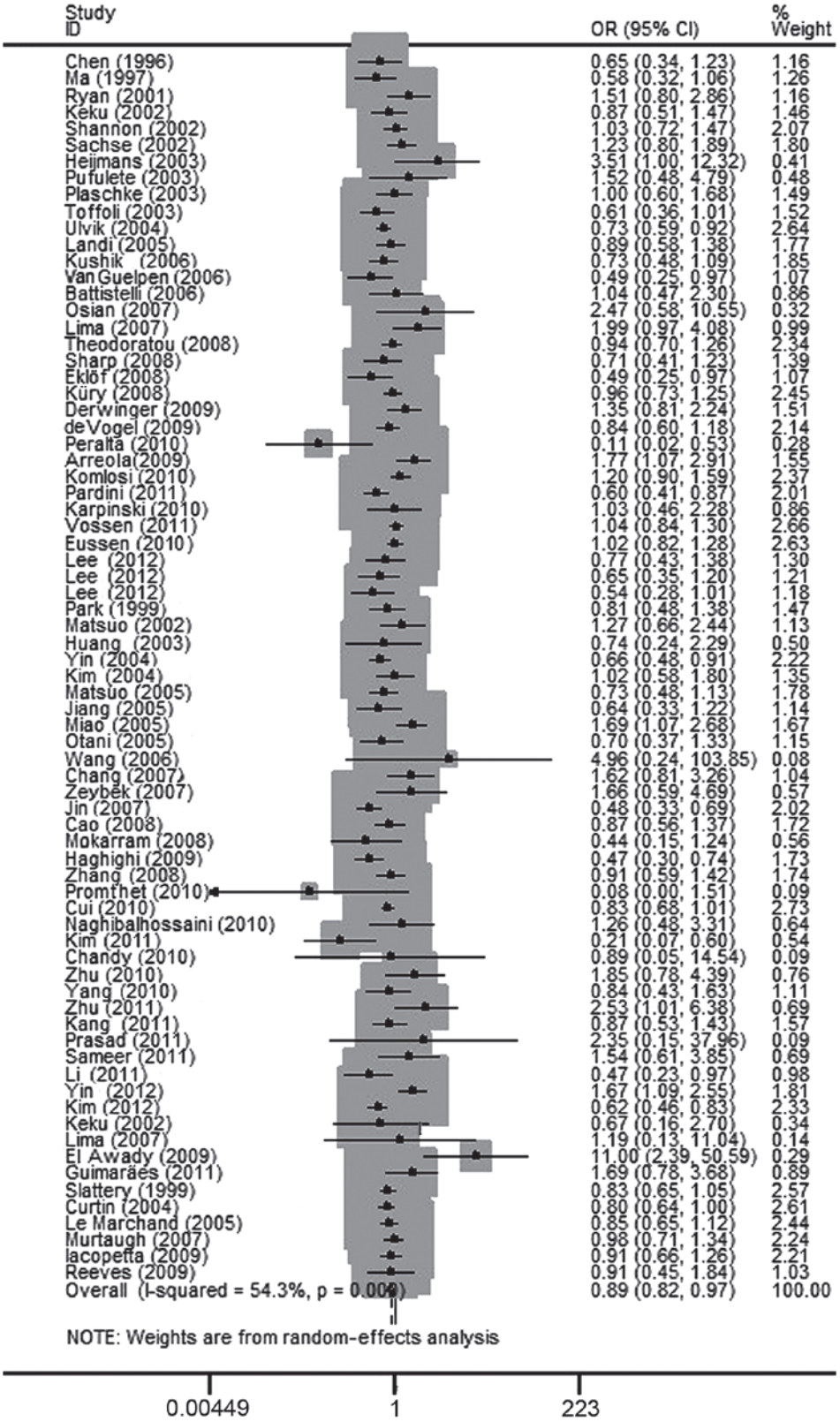

Figure 1. Forest plot for the association between methylenetetrahydrofolate reductase C677T polymorphism and colorectal cancer risk for the TT genotype compared to the CC genotype in the overall population. OR, odds ratio; CI, confidence interval.

test. The shapes of the funnel plot for each comparison indicated no obvious asymmetry (Figs. 3 and 4) and the Egger's test was then used to provide statistical evidence for the funnel plot symmetry. No significant publication bias was detected in the studies. The results are presented in Table III.

Discussion

The first study to evaluate the association between the MTHFR C677T polymorphism and CRC was conducted by Chen *et al* (10). The findings of that study suggested that the

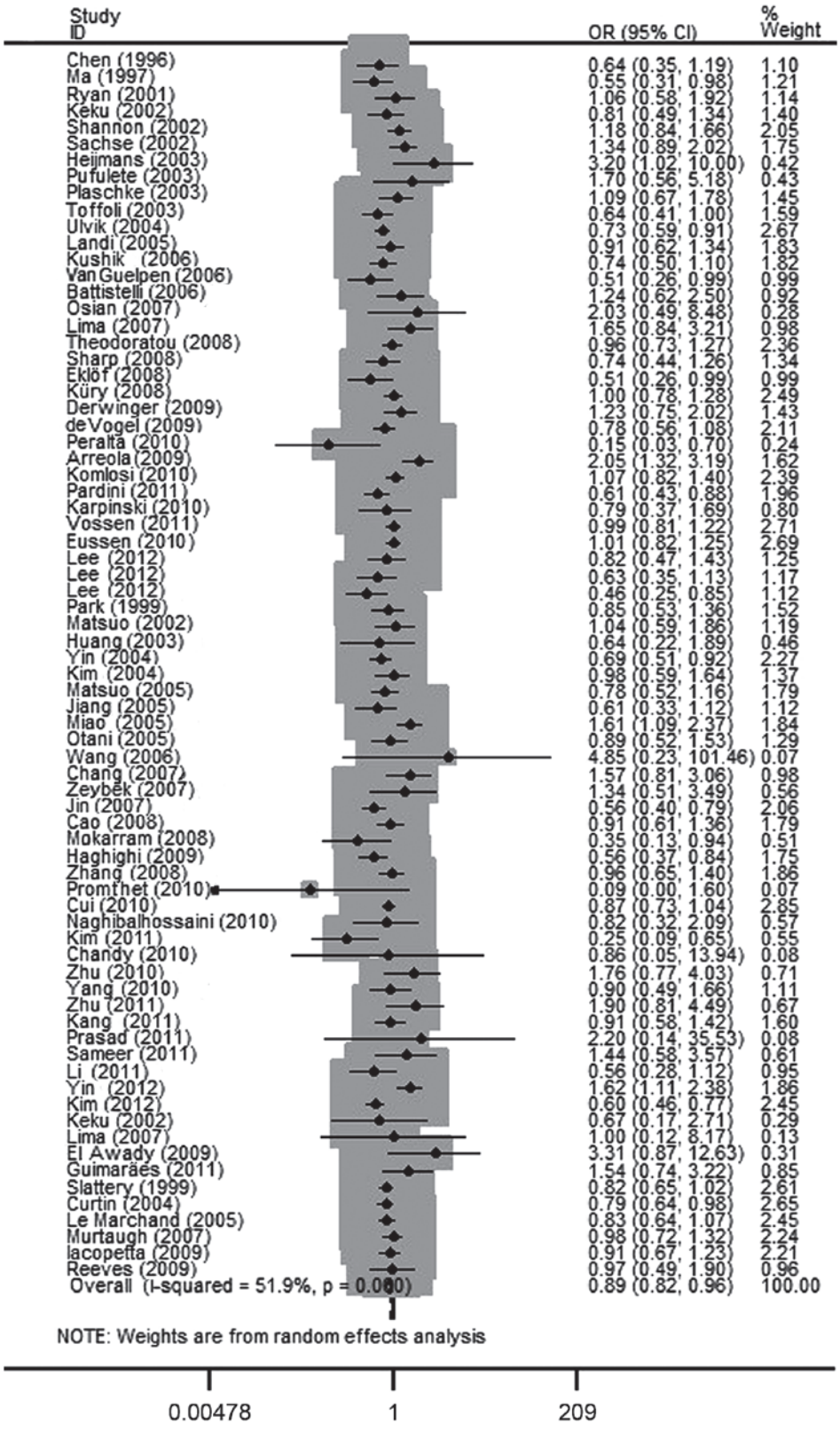

Figure 2. Forest plot for the association between methylenetetrahydrofolate reductase C677T polymorphism and colorectal cancer risk for the TT genotype compared to the CC+CT genotype in the overall population. OR, odds ratio; CI, confidence interval.

MTHFR C677T mutation affected enzyme activity and was involved in abnormal methylation as well as DNA synthesis, leading to colorectal tumorigenesis. Similar results were subsequently reported by Ma *et al* (16), Slattery *et al* (70) and Le Marchand *et al* (77). In addition, Le Marchand *et al* (77) observed that the MTHFR 1298C allele was weakly protective against CRC. However, Guimarães *et al* (69) reported that the carriers of the combined variants MTHFR 1298AC+CC and 677CT+TT exhibited an increased risk of CRC, whether in isolation or in combination. According to Shannon *et al* (14)

Table II. Association of MTHFR C677T and A1298C polymorphisms with CRC risk.

	TT vs. CC		CT vs. CC		TT vs. CC+CT		TT+CT vs. CC	
C677T	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
Total	0.89 (0.45-0.97)	0.009	1.02 (0.97-1.07)	0.378	0.89 (0.82-0.96)	0.003	1.00 (0.95-1.05)	0.889
Ethnicity								
European	0.90 (0.80-1.02)	0.088	1.02 (0.95-1.09)	0.661	0.90 (0.80-1.01)	0.075	0.99 (0.93-1.06)	0.831
Asian	0.86 (0.73-1.02)	0.087	1.03 (0.93-1.13)	0.583	0.87 (0.75-1.01)	0.059	1.00 (0.90-1.11)	0.981
African	1.96 (0.66-5.85)	0.225	1.75 (0.81-3.78)	0.153	1.49 (0.85-2.62)	0.165	1.80 (0.82.3.94)	0.143
Mixed	0.86 (0.76-0.96)	0.010	1.02 (0.95-1.10)	0.557	0.85 (0.76-0.95)	0.004	0.99 (0.92-1.06)	0.703
Tumor location								
Colon	0.86 (0.73-1.02)	0.079	1.08 (0.97-1.20)	0.161	0.83 (0.72-0.96)	0.014	1.03 (0.93-1.15)	0.566
Rectum	0.88 (0.73-1.07)	0.208	1.07 (0.98-1.16)	0.129	0.86 (0.77-0.97)	0.014	1.01 (0.89-1.15)	0.880
	CC vs. AA		AC vs. AA		CC vs. AA+AC		CC+AC vs. AA	
A1298C	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
Total	0.92 (0.81-1.05)	0.202	1.03 (0.98-1.08)	0.221	0.90(0.80-1.01)	0.086	1.02 (0.96-1.08)	0.619
Ethnicity								
European	1.01 (0.91-1.11)	0.864	1.04 (0.98-1.11)	0.169	0.99 (0.90-1.09)	0.841	1.04 (0.98-1.10)	0.208
Asian	0.69 (0.54-0.89)	0.004	1.00 (0.89-1.11)	0.984	0.69 (0.54-0.88)	0.002	0.96 (0.86-1.07)	0.466
African	1.92 (0.70-5.31)	0.207	1.33 (0.96-1.83)	0.082	1.50 (0.70-3.20)	0.294	1.45 (0.94-2.23)	0.091
Mixed	0.82 (0.69-0.98)	0.033	0.98 (0.88-1.10)	0.730	0.83 (0.70-0.99)	0.035	0.95 (0.85-1.05)	0.316
Tumor location								
Colon	0.81 (0.59-1.12)	0.200	1.03 (0.94-1.13)	0.535	0.81 (0.69-0.95)	0.012	0.99 (0.91-1.08)	0.872
Rectum	0.85 (0.50-1.42)	0.530	1.00 (0.79-1.26)	0.980	0.85 (0.55-1.32)	0.474	0.95 (0.74-1.23)	0.719

CRC, colorectal cancer; OR, odds ratio; CI, confidence interval.

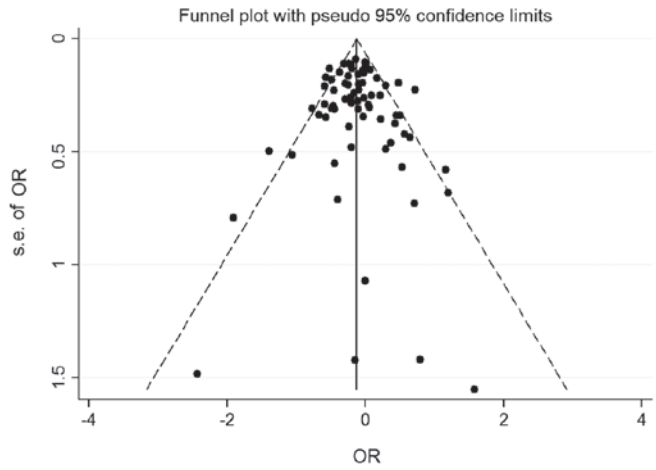

Figure 3. Funnel plot with pseudo 95% confidence limits on the association between methylenetetrahydrofolate reductase C677T polymorphism and colorectal cancer risk. SE, standard error; OR, odds ratio.

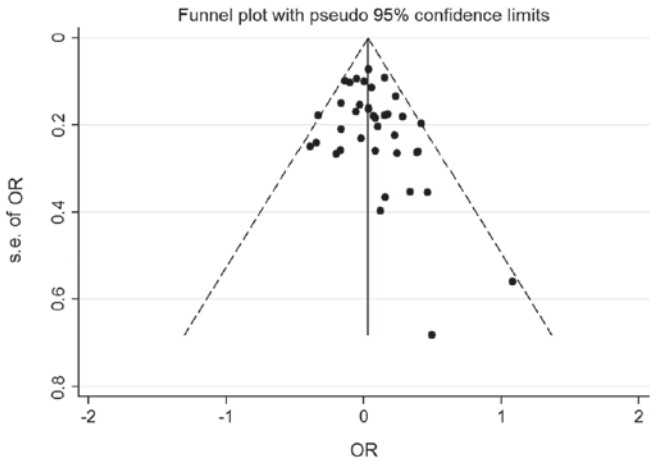

Figure 4. Funnel plot with pseudo 95% confidence limits on the association between methylenetetrahydrofolate reductase A1298C polymorphism and colorectal cancer risk. SE, standard error; OR, odds ratio.

and Prasad *et al* (63), the MTHFR polymorphism C677T was a risk factor for CRC development. Furthermore, several other published studies failed to support an effect of MTHFR gene polymorphisms on CRC risk (43,49,60), due to statistically non-significant results.

The conflicting conclusions among the studies mentioned above may be attributed to several causes. First, the sample sizes of the populations included in several studies were relatively small (19,27,69), which may result in false-positive or false-negative outcomes. Second, the eligibility criteria for inclusion of control subjects differed among the studies. Certain studies were hospital-based (35,45,50), whereas others were population-based (12,13,47,63). Therefore, some controls were non-cancer cases, whereas others were healthy individuals. The inclusion of individuals from different ethnic backgrounds should also be considered.

Table III. Results of Egger's test for the models.

Polymorphism	Model	T-value	P-value	95% CI of intercept value	
MTHFR C677T					
	TT vs. CC	1.04	0.303	-0.3457011	1.096597
	CT vs. CC	1.86	0.067	-0.0274857	0.7770219
	TT vs. CC+CT	0.62	0.535	-0.485652	0.9266581
	TT+CT vs. CC	1.66	0.102	-0.1176829	1.27792
MTHFR A1298C					
	CC vs. AA	-0.52	0.605	-0.9875751	0.5834467
	AC vs. AA	1.57	0.125	-0.1645624	1.29779
	CC vs. AA+AC	-1.02	0.312	-1.094435	0.3594053
	CC+AC vs. AA	1.13	0.266	-0.3669942	1.292081

CI, confidence interval.

As regards the conflicting results, we performed a meta-analysis to elucidate the association of MTHFR C677T and A1298C polymorphisms with CRC risk and to provide a comprehensive assessment. In this meta-analysis, the pooled results indicated that the homozygous variant of MTHFR C677T polymorphism exerted a protective effect against CRC development (OR=0.89; 95% CI: 0.82-0.97). However, when analysis was performed by ethnicity, this effect was not observed in all the subgroups, except for the mixed population (OR=0.86; 95%CI: 0.76-0.96). Additionally, a significant association was observed between MTHFR 1298CC and CRC in the mixed population (OR=0.82; 95% CI: 0.69-0.98). When this analysis was restricted by limiting studies to Asian populations, the MTHFR 1298CC genotype exhibited a decreased risk of CRC, with an OR of 0.69 (95% CI: 0.54-0.89). In the subanalysis by tumor location it was demonstrated that individuals with the MTHFR 677TT genotype exhibited a decreased risk of colon and rectal cancer, with an OR of 0.83 (95% CI: 0.72-0.96) and 0.86 (95% CI: 0.77-0.97), respectively. It was also confirmed that the MTHFR A1298C polymorphism is involved in colon cancer development (CC vs. AA+AC: OR=0.81; 95% CI: 0.69-0.96). Our findings in this meta-analysis were consistent with the results reported by the majority of the published studies.

According to Begg's funnel plots and Egger's test, there was no significant publication bias in the present meta-analysis. However, there was obvious heterogeneity, which was a potential problem when interpreting the results of the meta-analysis. Several sources of heterogeneity should be considered. First, different selection criteria of cases and controls, as well as gender and age distribution, may affect between-study heterogeneity. Second, there was some diversity among the studies regarding design, sample size and family history. Ethnic variations were also crucial as it was demonstrated that the heterogeneity was decreased when analyzed by ethnicity in this meta-analysis. The different ethnicities were distinguished according to geography; however, potential confounding factors, such as genetic background, lifestyle and dietary habits, could not be excluded.

The present study has certain limitations. First, the criteria for inclusion of controls differed among the studies. The controls in certain studies were selected from healthy individuals, whereas the controls in other studies were selected from non-cancer cases. Second, our results were based on unadjusted OR values, which may lead to relatively low power of the estimation of the real association. The gender and age distribution of the participants, the dietary pattern, the folate status, alcohol consumption and other risk factors may affect between-study heterogeneity. An analysis should be conducted to obtain adjusted ORs for other covariates, such as age, gender and folate status, provided more individual study data are available. Third, the subgroup analyses had insufficient statistical power to detect the association. The gene-gene and gene-environment interactions may affect the association between MTHFR polymorphisms and CRC. According to the study by Keku *et al* (12), the combination of MTHFR 677CC and 1298AA genotypes exhibited an increased risk of CRC. In addition, Ma *et al* (16) and Kim *et al* (67) demonstrated that a high folate intake was associated with reduced risk of CRC and high alcohol consumption was associated with an increased risk of CRC. The gene-gene and gene-environment interactions could not be examined due to unavailability of individual data.

Despite the limitations described above, our meta-analysis also has certain advantages. The substantial number of cases and controls were pooled from different studies, which provided more reliable evidence on the association between MTHFR polymorphisms and the risk of CRC. In addition, the study subjects were classified into colon and rectal cancer groups, in order to exclude certain confounding factors. The pooled data clearly demonstrated that MTHFR C677T significantly affected carcinogenesis in the colon and rectum, whereas A1298C appeared to be mainly associated with rectal tumorigenesis. This finding may be attributed to different carcinogenic mechanisms underlying colon and rectal cancer.

In conclusion, the results of this meta-analysis indicated a significant association of the MTHFR C677T and A1298C polymorphisms with the risk of CRC. Particularly, the MTHFR 677T and 1298C alleles were associated with a low risk of CRC.

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