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Research Article

Methylenetetrahydrofolate reductase C677T polymorphism and colorectal cancer susceptibility: a meta-analysis

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The association between methylenetetrahydrofolate reductase (*MTHFR*) C677T polymorphism and colorectal cancer (CRC) susceptibility has been researched in numerous studies. However, the results of these studies were controversial. Therefore, the objective of this meta-analysis was to offer a more convincible conclusion about such association with more included studies. Eligible studies published till May 1, 2017 were searched from PubMed, Embase, Web of Science, and CNKI database about such association. Pooled odds ratios (ORs) together with 95% confidence intervals (CIs) were calculated to evaluate such association. And the Begg's funnel plot and Egger's test were applied to assess the publication bias. This meta-analysis contained 37049 cases and 52444 controls from 87 publications with 91 eligible case–control studies. Because of lack of data for a particular genotype in several studies, all the included studies were analysed barely in the dominant model. Originally, there was no association between *MTHFR* C677T polymorphism and CRC susceptibility (OR =0.99, 95% CI =0.94–1.05). After excluding 13 studies according to their heterogeneity and publication bias, rs1801133 polymorphism was found to reduce the risks of CRC significantly (OR =0.96, 95% CI =0.94–0.99). In the subgroup analysis of ethnicity, there was a significant association in Asians (OR =0.94, 95% CI =0.89–1.00). Furthermore, when stratified by the source of controls and genotyping methods, the positive results were observed in population-based control group (OR =0.97, 95% CI =0.93–1.00) and PCR-restriction fragment length polymorphism (PCR-RFLP) method (OR =0.95, 95% CI =0.91–0.99. The results of the meta-analysis suggested that *MTHFR* C677T polymorphism was associated with CRC susceptibility, especially in Asian population.

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Introduction

Colorectal cancer (CRC) is a critical public health problem, which is the third most commonly diagnosed cancer and the third common cause of cancer deaths in both males and females. There were 134490 new CRC cases and 49190 mortalities by estimation in the United States in 2016 [1]. The colorectal carcinogenesis is a complex multistep progress (a benign adenomatous polyp – an advanced adenoma with high-grade dysplasia – an invasive cancer) with altered expression of oncogenes, tumor suppressor genes and DNA repair genes [2]. However, the etiology of CRC is still unclear. It is known to all that CRC is a multifactorial and multigenic disease, and is influenced by environment conditions, diet habits, genetic

mutations, and *Escherichia coli* infection [3,4]. With increasing numbers of studies, more gene polymorphisms were found to contribute to CRC [5]. These single nucleotide polymorphisms (SNPs) can be used as makers for improving cancer diagnosis and determination of treatment plans [6].

As a key enzyme and an important regulator for the metabolism of folate/vitamin B$_9$, methylenetetrahydrofolate reductase (*MTHFR*) catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate [7]. Simultaneously, the 5-methyltetrahydrofolate is the main circulatory form of folate in the body and provides a methyl group to convert the amino acid homocysteine into methionine, which is the precursor of S-adenosylmethionine (SAM). SAM is the major methyl donor in the cell and takes part in DNA methylation [8]. Therefore, *MTHFR* not only plays a role in making proteins and other important compounds, but also is an important factor in DNA methylation, synthesis, and repair [9]. The enzyme is encoded by the *MTHFR* gene located on the short arm of chromosome 1-1p36.3 [10]. Previously, several mutations of *MTHFR* gene have been found and *MTHFR* C677T (rs1801133) is the most common type amongst them. *MTHFR* C677T represents an alanine-to-valine substitution at nucleotide position 677 in exon 4 resulting in thermolability and concurrent decreased activity of the enzyme [11,12]. *MTHFR* gene mutations lead to *MTHFR* enzyme dificiency, low plasma folate levels, hyperhomocysteinemia [13,14] and certain diseases such as cardiovascular disease, pregnancy complications, neural defect, and several cancers including CRC [15-21]. With a growing number of studies conducted to explore such association, we hypothesized that rs1801133 was likely to relate to colorectal carcinogenesis.

Many researchers have carried out a large number of studies to examine the potential association between *MTHFR* C677T polymorphism and CRC susceptibility. But, the results are still inconclusive so far. Thus, the aim of this meta-analysis including all available case–control studies was to investigate a more reliable association.

Materials and methods

We searched several databases including PubMed, Embase, Web of Science, and CNKI database for published studies about exploring the association between *MTHFR* C677T polymorphism and CRC susceptibility till May 1, 2017. The search strategy included listed key words: 'methylenetetrahydrofolate reductase', '*MTHFR* polymorphism', 'C677T', 'rs1801133', and 'risk or susceptibility' and 'colorectal or colon or rectal cancer'. Furthermore, we manually searched the reference lists of clinical trials and former meta-analyses for more relevant studies. When duplicate data appeared in different publications, this meta-analysis only adopted the most recent study or the study with the most complete information. The meta-analysis was on the basis of the preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) [22]. The eligible studies needed to accord with the following inclusion criteria: (i) case–control studies; (ii) the language was not restricted to English; (iii) investigating the association between *MTHFR* C677T polymorphism and CRC susceptibility; (iv) offering enough raw data to calculate odds ratio (OR) with 95% confidence interval (CI). Additionally, exclusion criteria were as follows: (i) non-case–control studies; (ii) lack of sufficient data for calculating genotype frequency; (iii) case–control studies about examining the relationship between *MTHFR* C677T polymorphism and colorectal adenoma; (iv) duplicated publications.

Data extraction

In order to guarantee the accuracy of extracted information, two authors individually reviewed each publication and extracted useful data on the basis of the inclusion criteria listed above. When disagreements arose in the course of data extraction, discussion was carried out with other authors until the agreements were reached. The following information were extracted from each study to accomplish a standardized sheet: first author's name, year of publication, ethnicity of population, source of controls (hospital based or population based), genotyping method, sample size of cases and controls, genotype frequency of rs1801133 in cases and controls, and the results of the Hardy–Weinberg equilibrium (HWE) test.

Statistical analysis

The relationship between *MTHFR* C677T polymorphism and CRC susceptibility was analyzed by using five models including the dominant model (CT + TT compared with CC), the recessive model (TT compared with CT + CC), the homozygous model (TT compared with CC), the heterozygous model (CT compared with CC), and the allele model (T compared with C). The goodness-of-fit χ^2 test was conducted to evaluate the HWE in control groups and $P < 0.05$ was regarded as significant disequilibrium [23]. Stratified analysis were performed by ethnicity, source of controls, and genotyping method. Besides, the pooled OR together with 95% CI were measured to bring out the strength of such association. The fixed effects model (Mantel–Haenszel method) and the random effects model (Dersimonian–Laird method) were selected to use based on heterogeneity in the meta-analysis. If there was no or little heterogeneity,
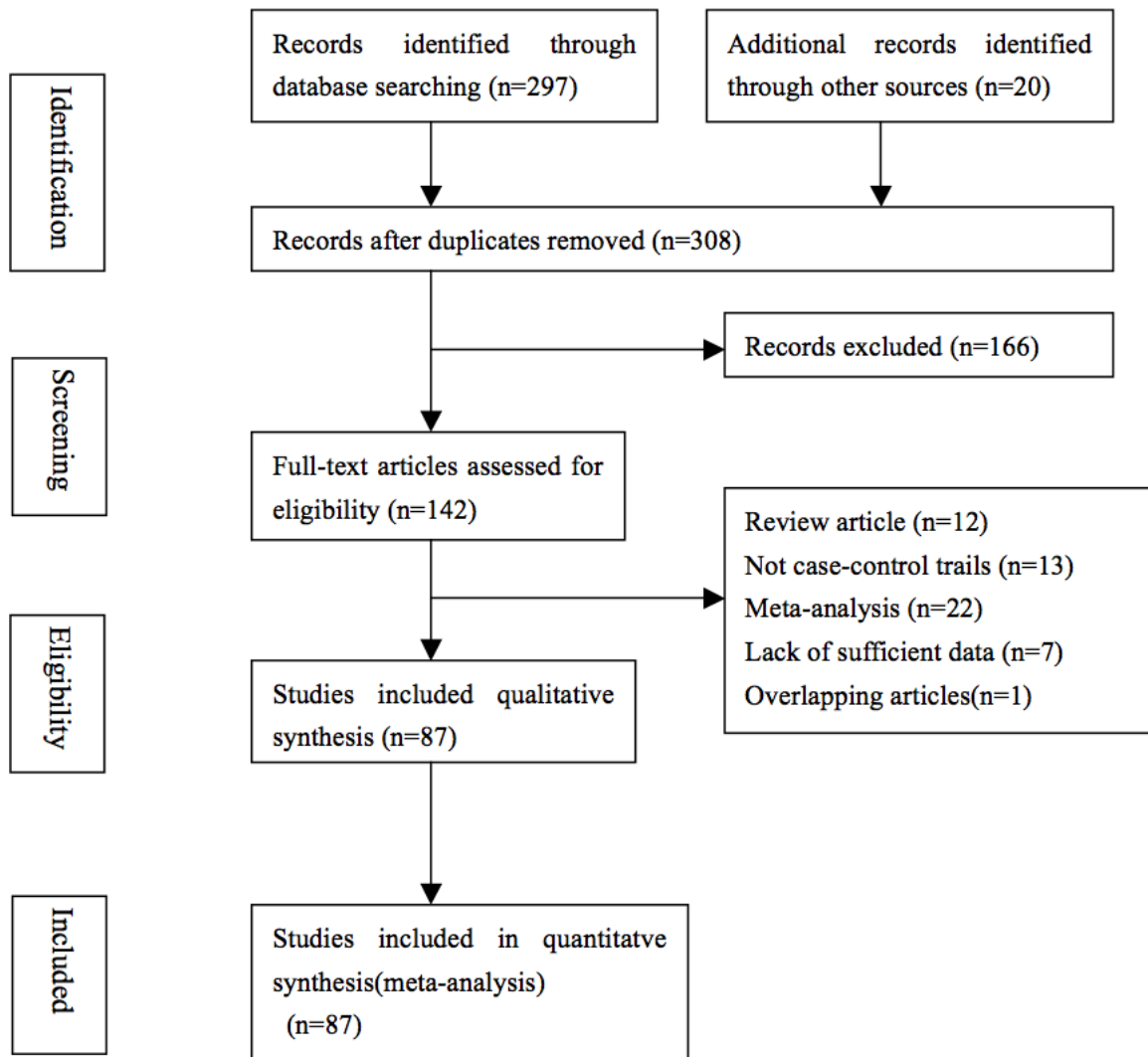
Figure 1. Flowchart of literature search and selection process

the fixed effects model was used; otherwise, the random effects model was used. Due to only particular genotypes extracted in several studies, the dominant model analysis were carried out for all the included studies [84]. Galbraith graph was performed to explore the impossible cause of heterogeneity [24]. A sensitivity analysis was conducted to assess the stability of the results. Begg's funnel plot was performed for potential publication bias and Egger's linear regression test was executed to assess funnel plot asymmetry statistically. If $P<0.05$, publication bias existed [25]. All statistical data analyses were carried out by using Stata software (version 12.0, StataCorp LP, College Station, TX, U.S.A.).

Results

Characteristics of the studies

According to PRISMA-P, this meta-analysis contained 37049 cases and 52444 controls that were combined from 87 publications with 91 eligible case–control studies to examine the relationship between rs1801133 polymorphism and CRC risks [26-112]. The literature retrieval and selection process are shown in the flowchart in Figure 1. Detailed information of each study were listed in Table 1. The distribution of genotypes in controls was consistent with HWE except 15 studies [33-35,37,39,47,63,71,76,80,87,88,106,110,111]. In these studies, four ethnicities of population were included: Asian, Caucasian, African, and mixed ethnic group. Nine genotyping methods were applied: PCR-restriction fragment length polymorphism (PCR-RFLP), real-time PCR (RT-PCR), PCR-single

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Table 1 Characteristics of individual studies included in the meta-analysis

MTHFR rs1801133 Year	Surname (References)	Ethnicity	SOC	Genotyping	Case	Control	Case (n) CC	CT	TT	Control (n) CC	CT	TT	HWE
2016	Haerian [26]	Asian	HB	Taqman	1123	1298	607	421	95	667	523	108	Y
2015	Kim [27]	Asian	PB	PCR-RFLP	477	514	159	248	70	172	265	77	Y
2014	Rai [28]	Asian	PB	PCR-RFLP	155	294	137	17	1	261	31	2	Y
2014	**Ozen** [29]	**Caucasian**	**PB**	**RT-PCR**	**86**	**212**	**36**	**32**	**18**	**207**	**5**	**0**	**Y**
2013	**Ashmore** [30]	**Caucasian**	**PB**	**RT-PCR**	**625**	**603**	**241**	**309**	**75**	**263**	**259**	**81**	**Y**
2013	**Delgado-Plasencia** [31]	**Caucasian**	**HB**	**PCR-RFLP**	**50**	**103**	**32**	**16**	**2**	**44**	**50**	**9**	**Y**
2013	Yousef [32]	Asian	PB	PCR-RFLP	128	116	79	45	4	59	45	12	Y
2012	Lee [33]	Caucasian	PB	Taqman	531	1004	250	229	52	464	391	149	N
2012	Promthet [34]	Asian	HB	PCR-RFLP	112	242	93	18	1	185	49	8	N
2012	Kim [35]	Asian	HB	Taqman	787	656	265	393	129	205	289	162	N
2012	Yin [36]	Asian	HB	RT-PCR	370	370	124	167	79	139	178	53	Y
2011	Sameer [37]	Asian	PB	PCR-RFLP	86	160	59	18	9	121	27	12	N
2011	Vossen [38]	Caucasian	PB	Taqman	1762	1811	737	823	202	795	807	209	Y
2011	Kang [39]	Asian	PB	PCR-RFLP	255	448	87	134	34	145	238	65	N
2011	**Zhu** [40]	**Asian**	**PB**	**PCR-RFLP**	**86**	**100**	**29**	**42**	**15**	**49**	**41**	**10**	**Y**
2011	Pardini [41]	Caucasian	HB	PCR-RFLP	666	1376	317	307	42	613	627	136	Y
2011	Kim [42]	Asian	HB	MSP	67	53	30	30	7	15	21	17	Y
2011	**Prasad** [43]	**Asian**	**PB**	**PCR-RFLP**	**110**	**241**	**97**	**12**	**1**	**228**	**12**	**1**	**Y**
2011	Li [44]	Asian	PB	PCR-RFLP	137	145	68	54	15	55	64	26	Y
2011	Jokic [45]	Caucasian	PB	Taqman	300	300	139	130	31	142	130	28	Y
2011	Guimaracs(a) [46]	Caucasian	HB	PCR-RFLP	101	188	42	44	15	92	79	17	Y
2011	Guimaracs(b) [46]	African	HB	PCR-RFLP	12	188	6	6	0	92	79	17	Y
2010	**Komlosi** [47]	**Caucasian**	**PB**	**PCR-RFLP**	**951**	**939**	**398**	**427**	**126**	**442**	**380**	**117**	**N**
2010	**Karpinski** [48]	**Caucasian**	**HB**	**MSP**	**186**	**140**	**74**	**97**	**15**	**71**	**55**	**14**	**Y**
2010	Cui [49]	Asian	PB	PCR-RFLP	1829	1700	622	923	284	540	863	297	Y
2010	Eussen [50]	Caucasian	PB	MALDI-TOF-MS	1329	2366	567	608	154	1019	1076	271	Y
2010	Chandy [51]	Asian	HB	PCR-RFLP	100	86	74	25	1	66	19	1	Y
2010	**Naghibalhossaini** [52]	**Asian**	**PB**	**MS-PCR**	**151**	**231**	**64**	**80**	**7**	**150**	**68**	**13**	**Y**
2010	Promthet [53]	Asian	HB	PCR-RFLP	130	130	104	26	0	94	31	5	Y
2010	Yang [54]	Asian	PB	Sequenom	141	165	58	61	22	62	75	28	Y
2010	**Fernández-Peralta** [55]	**Caucasian**	**HB**	**PCR-RFLP**	**143**	**103**	**89**	**52**	**2**	**44**	**50**	**9**	**Y**
2010	Zhu [56]	Asian	PB	PCR-RFLP	216	111	88	102	26	50	53	8	Y
2009	Vogel [57]	Caucasian	PB	RT-PCR	689	1793	318	320	51	876	750	167	Y
2009	Iacopetta [58]	Mixed	PB	PCR-SSCP	850	958	382	386	82	428	429	101	Y
2009	Arreola [59]	Caucasian	PB	PCR-RFLP	369	170	124	126	119	59	79	32	Y
2009	Reeves [60]	Caucasian	HB	Taqman	206	211	105	83	18	101	91	19	Y
2009	**Awady** [61]	**African**	**HB**	**PCR-RFLP**	**35**	**68**	**6**	**23**	**6**	**44**	**20**	**4**	**Y**
2009	Derwinger [62]	Caucasian	PB	Taqman	544	299	273	216	55	167	107	25	Y
2008	**Haghighi** [63]	**Asian**	**HB**	**PCR/pyrosequencing**	**234**	**257**	**117**	**68**	**49**	**94**	**80**	**83**	**N**
2008	Sharp [64]	Caucasian	PB	PCR-RFLP	251	394	117	111	23	170	177	47	Y
2008	Kury [65]	Caucasian	PB	Taqman	1023	1121	435	452	136	457	515	149	Y
2008	Mokarram [66]	Asian	HB	MSP	151	81	64	80	7	40	31	10	Y
2008	Cao [67]	Asian	PB	PCR-RFLP	315	370	109	154	52	121	183	66	Y
2008	Theodoratou [68]	Caucasian	PB	MassARRAY	999	1010	447	441	111	439	455	116	Y
2008	Ekolf [69]	Caucasian	PB	Taqman	220	414	123	85	12	212	160	42	Y
2008	Zhang [70]	Asian	HB	PCR-RFLP	300	299	97	136	67	91	139	69	Y
2008	Guerreiro [71]	Caucasian	HB	Taqman	196	200	94	76	26	84	107	9	N
2007	Osian [72]	Caucasian	HB	PCR-RFLP	69	67	38	25	6	47	17	3	Y
2007	Zeybek [73]	Asian	HB	PCR-RFLP	52	144	18	27	7	64	65	15	Y
2007	Lima(a) [74]	Caucasian	HB	PCR-RFLP	90	300	36	40	14	143	127	30	Y

Table 1 Characteristics of individual studies included in the meta-analysis (Continued)

Year	Surname (References)	Ethnicity	SOC	Genotyping	Case	Control	Case (n) CC	Case (n) CT	Case (n) TT	Control (n) CC	Control (n) CT	Control (n) TT	HWE
2007	Lima(b) [74]	African	HB	PCR-RFLP	10	300	4	5	1	143	127	30	Y
2007	Chang [75]	Asian	HB	RT-PCR	195	195	85	86	24	92	87	16	Y
2007	Murtaugh [76]	Mixed	PB	PCR-RFLP	742	970	357	301	84	466	392	112	N
2007	**Jin [77]**	**Asian**	**PB**	**Taqman**	**449**	**672**	**182**	**211**	**56**	**211**	**325**	**136**	**Y**
2007	Curtin [78]	Mixed	PB	PCR-RFLP	916	1972	432	402	82	887	858	227	Y
2007	Hubner [79]	Caucasian	PB	Taqman	1685	2691	743	759	183	1173	1192	326	Y
2006	Koushik [80]	Caucasian	PB	Taqman	349	794	166	145	38	355	327	112	N
2006	Battistelli [81]	Caucasian	HB	PCR-RFLP	93	100	32	40	21	30	51	19	Y
2006	Van Guelpen [82]	Caucasian	PB	Taqman	220	415	123	85	12	212	161	42	Y
2006	Wang [83]	Asian	PB	PCR-RFLP	302	291	257	43	2	255	36	0	Y
2006	Chen [84]	Asian	PB	PCR-RFLP	138	340	52	86		133	207		-
2005	Matsuo [85]	Asian	HB	PCR-RFLP	256	771	106	114	36	289	348	134	Y
2005	Landi [86]	Caucasian	HB	RT-PCR	350	309	128	158	64	109	139	61	Y
2005	Marchand [87]	Mixed	PB	PCR-RFLP	817	2021	394	336	87	987	779	255	N
2005	Jiang [88]	Asian	PB	PCR-RFLP	125	339	51	59	15	134	143	62	N
2005	Otani [89]	Asian	HB	MassARRAY	106	222	32	49	25	51	114	57	Y
2005	Miao [90]	Asian	PB	PCR-RFLP	198	420	53	87	58	133	201	86	Y
2004	Kim [91]	Asian	HB	PCR-RFLP	243	225	86	122	35	83	109	33	Y
2004	Ulvik [92]	Caucasian	PB	Taqman	2159	2190	1103	899	157	1092	886	212	Y
2004	Yin [93]	Asian	PB	PCR-RFLP	685	778	270	330	85	278	367	133	Y
2004	Curtin [94]	Mixed	HB	PCR-RFLP	1608	1972	734	724	150	887	858	227	Y
2003	Pufulete [95]	Caucasian	HB	PCR-RFLP	28	76	16	6	6	41	29	6	Y
2003	Plaschke [96]	Caucasian	PB	PCR-RFLP	287	346	133	120	34	149	159	38	Y
2003	Toffoli [97]	Caucasian	PB	PCR-RFLP	276	279	93	145	38	83	140	56	Y
2003	Heijmans [98]	Caucasian	PB	PCR-RFLP	18	793	7	7	4	399	329	65	Y
2003	Huang [99]	Asian	HB	PCR-RFLP	82	82	36	40	6	40	33	9	Y
2003	Barna [100]	Caucasian	PB	PCR-RFLP	101	196	46	48	7	84	97	15	Y
2002	Keku(a) [101]	Caucasian	PB	Taqman/PCR-PFLP	308	539	144	140	24	265	223	51	Y
2002	Keku(b) [101]	African	PB	Taqman/PCR-PFLP	244	329	198	43	3	264	59	6	Y
2002	Marchand(a) [102]	Caucasian	PB	PCR-RFLP	149	171	66	64	19	66	81	24	Y
2002	Marchand(b) [102]	Asian	PB	PCR-RFLP	399	485	170	180	49	191	214	80	Y
2002	Shannon [103]	Caucasian	PB	PCR-SSCP/RFLP	501	1207	249	197	55	533	560	114	Y
2002	Matsuo [104]	Asian	HB	PCR-RFLP	142	241	39	81	22	81	124	36	Y
2002	Sachse [105]	Caucasian	PB	PCR-RFLP	490	592	238	199	53	271	272	49	Y
2002	Chen [106]	Caucasian	PB	PCR-RFLP	202	326	92	92	18	145	132	49	N
2001	**Ryan**	**Caucasian**	**PB**	**PCR-RFLP**	**136**	**848**	**49**	**73**	**14**	**439**	**326**	**83**	**Y**
2000	Slattery [108]	Caucasian	PB	PCR-RFLP	232	164	106	107	19	73	71	20	Y
1999	Slattery [109]	Mixed	PB	PCR-RFLP	1467	1821	673	655	139	827	787	207	Y
1999	Park [110]	Asian	PB	PCR-RFLP	200	460	65	107	28	140	246	74	N
1997	Ma [111]	Caucasian	PB	PCR-RFLP	202	326	92	92	18	145	132	49	N
1996	Chen [112]	Caucasian	PB	PCR-RFLP	144	627	67	64	13	280	263	84	Y

These 13 studies in bold were removed afterward because of its heterogeneity and publication bias. Abbreviations: HB: hospital-based control; PB, population-based control; SOC, source of control.

strand conformation polymorphism (PCR-SSCP), methylation-specific PCR (MS-PCR), mutagenically separated PCR (MSP), MALDI-TOF-MS, Taqman, MassARRAY, and Sequenom. Depending on different sources of control, population-based and hospital-based control groups were distinguished in all the included studies.

Results of quantitative synthesis

Initially, there was no association between *MTHFR* C677T polymorphism and CRC susceptibility in the dominant model (OR =0.99, 95% CI =0.94–1.05). 0.94–1.05). Nevertheless, for the sake of looking for possible reasons that

might lead to such result, we performed heterogeneity analysis and tested publication bias. According to these results, 13 studies were excluded [29-31,40,43,47,48,52,55,61,63,77,107], the *P*-value was estimated to be 0.824, and the fixed effect model was applied. Ultimately, the results demonstrated that the rs1801133 polymorphism was significantly correlated with the risk of CRC (Figure 2) (dominant model: OR =0.96, 95% CI =0.94–0.99; recessive model: OR =0.90, 95% CI =0.83–0.96; homozygous model: OR =0.88, 95% CI =0.82–0.95; allele model: OR =0.95, 95% CI =0.93–0.98). All detailed results in the present meta-analysis are shown in Table 2.

In the subgroup analysis of ethnicity, *MTHFR* C677T polymorphism was found to reduce CRC susceptibility in Asians significantly (dominant model: OR =0.94, 95% CI =0.89–1.00 (Figure 3A); recessive model: OR =0.88, 95% CI =0.77–1.00; homozygous model: OR =0.86, 95% CI =0.75–1.00; allele model: OR =0.92, 95% CI =0.88–1.00). Simultaneously, significantly reduced risks were also found in mixed group (recessive model: OR =0.83, 95% CI =0.75–0.92; homozygous model: OR =0.84, 95% CI =0.75–0.93; allele model: OR =0.95, 95% CI =0.90–0.99). Amongst Caucasians, yet significantly reduced risks were only observed in the allele model (OR =0.96, 95% CI =0.93–1.00). Nevertheless, no significant associations were detected in Africans for all genetic models. When stratified by the source of controls, the positive results were observed in population-based control group (dominant model: OR =0.97, 95% CI =0.93–1.00 (Figure 3B); recessive model: OR =0.88, 95% CI =0.81–0.95; homozygous model: OR =0.87, 95% CI =0.80–0.93; allele model: OR =0.95, 95% CI =0.92–0.98). The similar significant associations were absent from hospital-based group for all the genetic models. The stratified analysis by genotyping methods showed that PCR-RFLP method (dominant model: OR =0.95, 95% CI =0.91–0.99 (Figure 3C); recessive model: OR =0.90, 95% CI =0.81–0.99; homozygous model: OR =0.88, 95% CI =0.79–0.97; allele model: OR =0.95, 95% CI =0.91–0.99) and Taqman method (recessive model: OR =0.86, 95% CI =0.73–1.00; homozygous model: OR =0.85, 95% CI =0.74–0.99; allele model: OR =0.94, 95% CI =0.89–0.99) were significantly correlated with risks of decreased CRC. However, RT-PCR method was not relevant to significant associations for all genetic models. In conclusion, the present meta-analysis suggested that *MTHFR* C677T polymorphism was connected with CRC susceptibility.

Test of heterogeneity

Heterogeneity analysis was performed in this meta-analysis, and heterogeneity was significantly observed between all the included studies in the dominant model (I^2 =62.0%, $P<0.001$; Figure 4A). In addition, the Galbraith radial plot illustrated heterogeneity obviously. Meanwhile, it specifically pointed out 13 studies that might have led to the obvious heterogeneity and insignificant results of the meta-analysis [27-29,38,41,45,46,50,53,59,61,75,105]. After excluding 13 studies, the heterogeneity decreased significantly (I^2 =0.0%, $P=0.789$; Figure 4B) in the present meta-analysis.

Publication bias

The Begg's funnel plot and Egger's test were performed to assess the publication bias. Initially, the Begg's funnel plot was asymmetrical obviously with all the included studies and it suggested a potential publication bias (Begg's test: $P=0.103$; Egger's test: $P=0.058$; Figure 5A). After the removal of 13 studies mentioned above [27-29,38,41,45,46,50,53,59,61,75,105], the plots seemed to have a symmetrical distribution in the funnel plot and then Egger's test was used to provide statistical evidence (Begg's test: $P=0.369$; Egger's test: $P=0.136$; Figure 5B). No significant publication bias was observed in the present studies.

Sensitivity analysis

In order to distinguish the impact of each study on the pooled ORs, we conducted one-way sensitivity analysis. Each time one study was omitted, meta-analysis was repeated and the statistical significance of the results was not changed. Therefore, the results confirmed that the present meta-analysis was relatively stable and reliable.

Discussion

MTHFR is a key enzyme in the folate metabolism and may play a role in the CRC carcinogenesis. It is an essential enzyme in the catalytic reaction that converts 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate. On one hand, 5,10-methylenetetrahydrofolate takes part in the thymidylate synthesis. On the other hand, 5-methyltetrahydrofolate promotes methionine synthesis and SAM-mediated methylations. In brief, *MTHFR* has an influence on DNA synthesis, methylation, and repair [113]. The *MTHFR* polymorphisms result in the decreased enzyme activity and then low levels of plasma folate and high homocysteine come to light. Folate is one of water-soluble B vitamins that takes part in various biochemical reactions with its activity to provide or accept one-carbon units [13]. Folate deficiency is likely to contribute to the development of CRC, and several mechanisms may explain how it leads to CRC, including DNA strand breaks, abnormal DNA methylation, and impaired DNA repair [114].
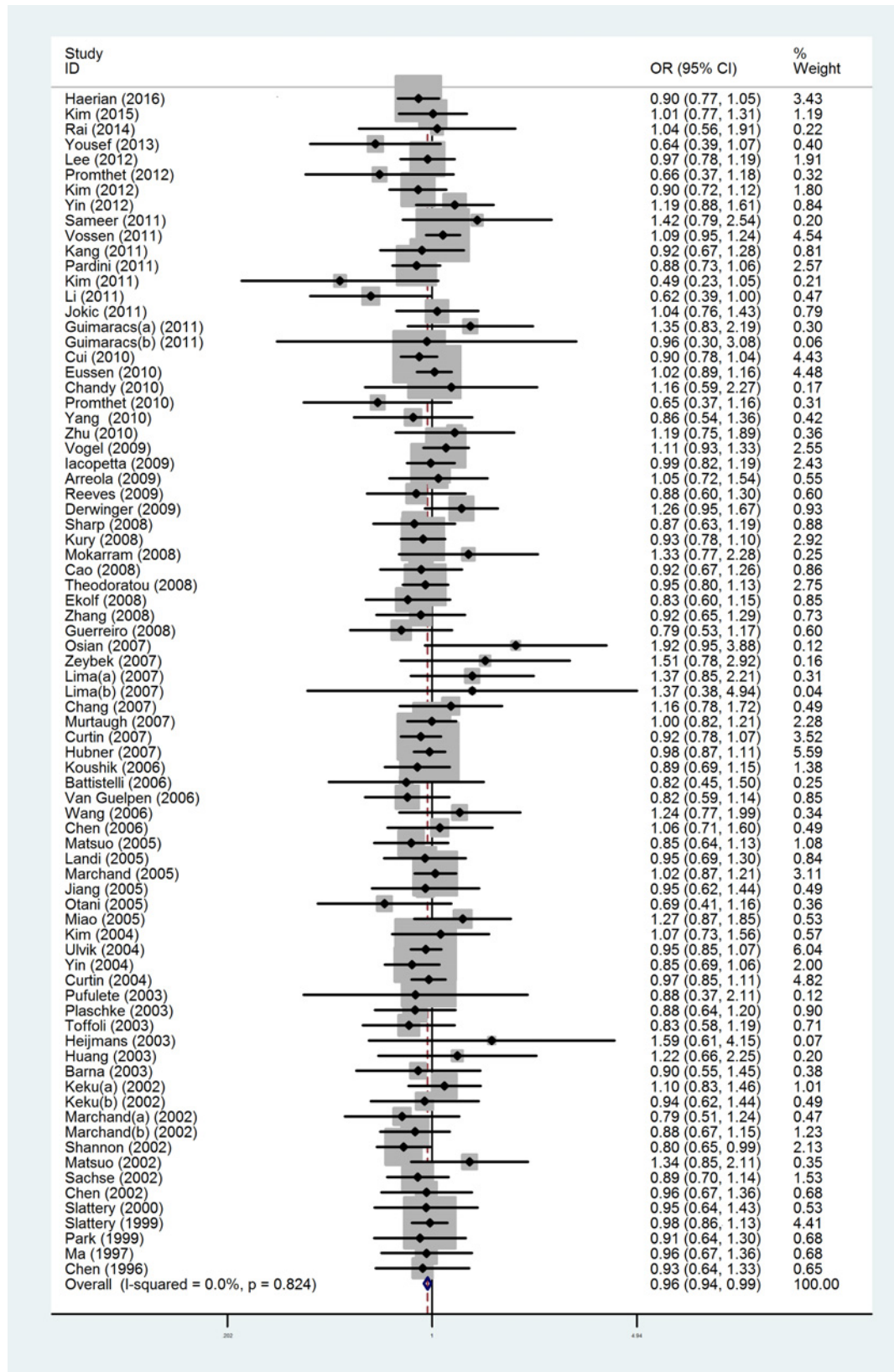
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Figure 2. Forest plots of the association between *MTHFR* C677T polymorphism and CRC susceptibility in dominant model after omitting these 13 studies with heterogeneity and publication bias

Table 2 Meta-analysis results for the included studies of the association between MTHFR rs1801133 polymorphism and risk of CRC

Variables	Number of studies	Dominant model			Recessive model			Homozygous model			Heterozygous model			Allele model		
		OR (95% CI)	P-values	I-squared (%)	OR (95% CI)	P-values	I-squared (%)	OR (95% CI)	P-values	I-squared (%)	OR (95% CI)	P-values	I-squared (%)	OR (95% CI)	P-values	I-squared (%)
		(CT + TT) compared with CC			TT compared with (CT + CC)			TT compared with CC			CT compared with CC			T compared with C		
rs1801133C>T																
All	78	**0.96 (0.94–0.99)**	**0.824**	**0.0**	**0.90 (0.83–0.96)**	<0.001	**49.9**	**0.88 (0.82–0.95)**	<0.001	**42.5**	0.99 (0.96–1.02)	0.950	0.0	**0.95 (0.93–0.98)**	**0.006**	**31.2**
Ethnicity																
Asian	33	**0.94 (0.89–1.00)**	**0.418**	**3.0**	**0.88 (0.77–1.00)**	0.001	**51.2**	**0.86 (0.75–1.00)**	0.001	**49.2**	0.96 (0.91–1.02)	0.933	0.0	**0.94 (0.88–1.00)**	0.002	**47.9**
Caucasian	36	0.97 (0.93–1.01)	0.711	0.0	0.93 (0.83–1.04)	<0.001	57.8	0.91 (0.82–1.01)	0.001	47.7	0.99 (0.95–1.03)	0.505	0.0	**0.96 (0.93–1.00)**	**0.079**	**26.2**
African	3	0.98 (0.67–1.42)	0.866	0.0	0.69 (0.24–2.03)	0.873	0.0	0.72 (0.24–2.15)	0.837	0.0	1.02 (0.69–1.51)	0.852	0.0	0.93 (0.67–1.30)	0.816	0.0
Mixed	6	0.98 (0.92–1.04)	0.959	0.0	**0.83 (0.75–0.92)**	**0.829**	**0.0**	**0.84 (0.75–0.93)**	**0.830**	**0.0**	1.02 (0.95–1.09)	0.967	0.0	**0.95 (0.90–0.99)**	**0.908**	**0.0**
Source of control																
HB	28	0.96 (0.90–1.03)	0.357	7.2	0.97 (0.81–1.16)	<0.001	59.6	0.96 (0.80–1.15)	<0.001	54.4	0.98 (0.92–1.04)	0.550	0.0	0.97 (0.90–1.05)	0.007	44.4
PB	50	**0.97 (0.93–1.00)**	**0.911**	**0.0**	**0.88 (0.81–0.95)**	**0.001**	**43.3**	**0.87 (0.80–0.93)**	**0.012**	**34.1**	0.99 (0.96–1.03)	0.970	0.0	**0.95 (0.92–0.98)**	**0.087**	**22.4**
Geotyping																
Taqman	14	0.96 (0.92–1.01)	0.568	0.0	**0.86 (0.73–1.00)**	<0.001	**65.0**	**0.85 (0.74–0.99)**	**0.004**	**57.3**	0.99 (0.94–1.05)	0.460	0.0	**0.94 (0.89–0.99)**	**0.085**	**36.4**
PCR-RFLP	50	**0.95 (0.91–0.99)**	**0.886**	**0.0**	**0.90 (0.81–0.99)**	**0.001**	**43.6**	**0.88 (0.79–0.97)**	**0.005**	**37.5**	0.98 (0.94–1.03)	0.992	0.0	**0.95 (0.91–0.99)**	**0.027**	**30.0**
RT-PCR	4	1.10 (0.97–1.26)	0.746	0.0	1.12 (0.76–1.64)	0.017	70.4	1.15 (0.79–1.66)	0.042	63.4	1.11 (0.96–1.27)	0.771	0.0	1.08 (0.95–1.22)	0.207	34.2

These 13 studies by Ozen et al., Ashmore et al., Delgado-Plasencia et al., Zhu et al., Prasad et al., Komlosi et al., Karpinski et al., Naghibalhossaini et al., Fernández-Peralta et al., Awady et al., Haghighi et al., Jin et al., Ryan et al. were removed [29, 30, 31, 40, 43, 47, 48, 52, 55, 61, 63, 77, 107].
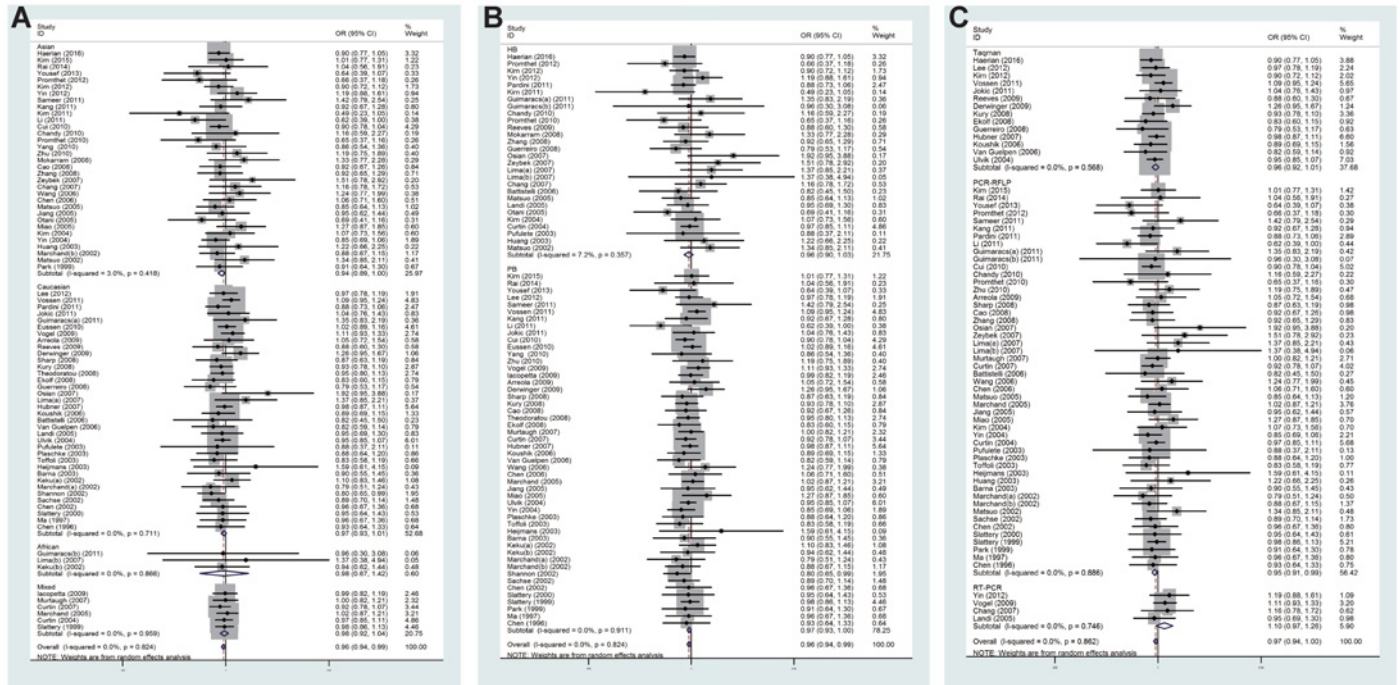
Figure 3. Forest plots of subgroup analysis of the association between *MTHFR* C677T polymorphism and CRC susceptibility in dominant model
(**A**) Stratified by ethnicity; (**B**) stratified by source of controls; (**C**) stratified by genotyping method.
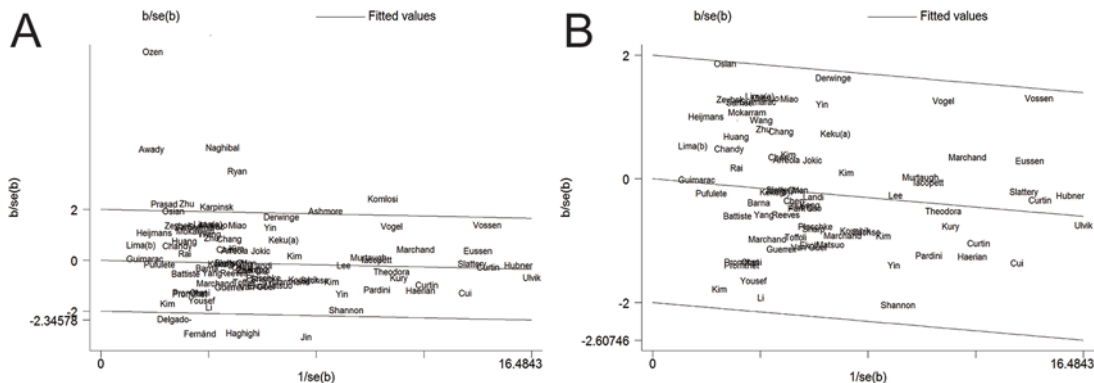

Figure 4. Galbraith plot of the association between *MTHFR* C677T polymorphism and CRC susceptibility in dominant model
(**A**) Stratified by ethnicity; (**B**) stratified by source of controls; (**C**) stratified by genotyping method.
(**A**) Before removing these 13 studies. (**B**) After the exclusion of these studies.

Several polymorphisms have been reported about the *MTHFR* gene coding relevant enzyme, and *MTHFR* C677T polymorphism is the most common one. Heretofore, various studies conducted to detect such association and obtained inconsistent results. Chen et al. [112], first reported that *MTHFR* variant homozygous (TT) genotype was closely linked to reduced incidence of CRC with low consumption of alcohol. In the next few years, similar results were replicated by several other studies [109-111]. However, another study of a homogeneous northern European population obtained different conclusions that *MTHFR* CT heterozygote had a significantly increased risk of developing CRC and no increased cancer risk was observed in TT homozygotes [107]. In addition, a hospital-based case–control study conducted by Matsuo et al. [104] found no significant relativity between *MTHFR* C677T and the
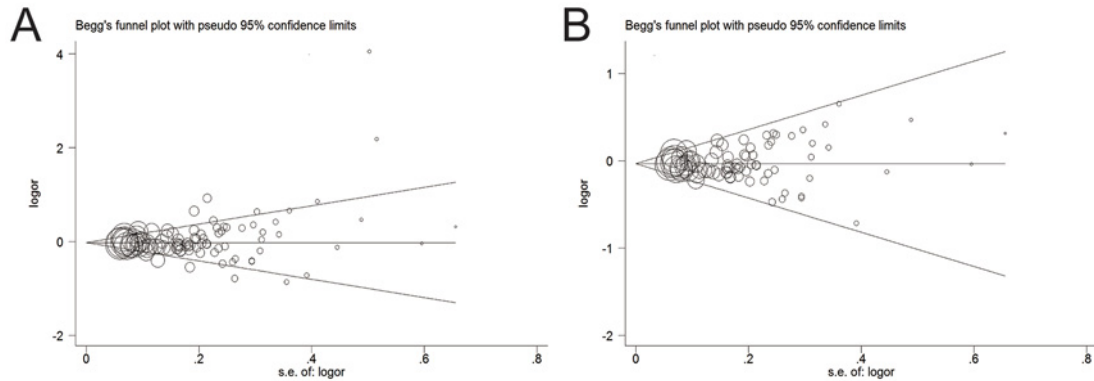
Figure 5. Begg's funnel plot of publication bias test
(**A**) Before omitting these 13 studies. (**B**) After the exclusion of these studies.

risks of CRC. Owing to the difference in study design and the sample size, the different ethnicity, and the diverse stratification, these controversial results were found in published studies. Hence, meta-analysis is essential to be carried out by combining all studies that meet the requirements to get more precise conclusions.

In recent years, there were several meta-analyses performed to elucidate the association of *MTHFR* C677T polymorphism and the susceptibility to CRC before [26,115-118]. Compared with them, this meta-analysis included the most eligible reported studies with the largest sample size and had no restrictions in ethnicity. Since the quality of included documents were disequilibrium, our initial analysis achieved no significant results with all eligible studies. In order to obtain more reliable results, the final conclusion were obtained excluding 13 studies in accordance with the analysis of heterogeneity and publication bias. In this meta-analysis, the pooled conclusions revealed that rs1801133 polymorphism significantly reduced the risk of CRC in the dominant model. The findings agreed with the overwhelming majority results reported by the published studies.

When stratified by ethnicity, there was a significant association with reduced risks of CRC in Asians. The result was consistent with the two previous meta-analysis based on the Asians [116,117]. Zhong et al. [118], carried out a meta-analysis obtaining similar results in East Asians and further subgroup analyses by country identified such association in Korea and Japan. Nevertheless, the recent meta-analysis failed to identify that rs1801133 polymorphism was connected with CRC susceptibility in Iranian population [26]. By means of stratified analysis based on the source of controls and genotyping methods, the positive results were observed in population-based control group and PCR-RFLP method. In general, the source of controls included healthy individuals and patients without CRC. Since the risks of CRC varies amongst individuals over a few years, it might have an impact on the results of relevant studies and make them unreliable. Therefore, inclusion criteria should be improved and studies with large sample sizes should be accepted. In the subgroup of genotyping method, there were nine methods applied for genotyping such as PCR-RFLP, RT-PCR, PCR-SSCP, MS-PCR, MSP, MALDI-TOF-MS, Taqman, MassARRAY, and Sequenom in the including studies. Specific methods and steps were described in each article. Amongst these 87 studies, the majority method was PCR-RFLP. Different methods have their own merits, and when all included studies used the same method, the final results would be more reliable.

In the present meta-analysis, we had obtained weak associations significantly with a large sample size. However, the potential limitations of the meta-analysis should be acknowledged. First, this meta-analysis was based on unadjusted effect estimates and 95% CI, and the influence of multiple cofactors such as age, gender, diet habits including intake of alcohol and consumption of cigarette, the level of folate, and the other environmental factors should be taken into consideration. Second, because of incomplete data of some genotypes, only the dominant model was analyzed in all the included studies. Third, we did not perform stratification analysis by serum folate levels, locations of the tumor and so on, which might result in confounding bias. In addition, after excluding 13 studies according to the analysis of heterogeneity and publication bias, the heterogeneity decreased significantly and the publication bias seemed to disappear. However, the selection bias existed because all the studies were published. Furthermore, the gene–gene and gene–environment interactions were not mentioned in this meta-analysis. In addition, the potential roles of the gene polymorphism which were hidden or magnified by other interactions were omitted.

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Conclusion

In summary, the present meta-analysis revealed that there was a significant association between *MTHFR* C677T polymorphism and susceptibility to CRC. Simultaneously, the TT genotype of *MTHFR* C677T polymorphism could reduce the risk of CRC. In addition, the associated risk of CRC was also reduced in Asians and those studies with population-based controls and used the PCR-RFLP method. Therefore, detection of the *MTHFR* C677T polymorphism might be used as markers for CRC prediction and treatment selection.

Competing interests

The authors declare that there are no competing interests associated with the manuscript.

Author contribution

Y.G., H.Y., and Z.Q. were responsible for conception and design. Y.G., H.Y., and F.W. provided the administrative support. S.S., Z.Q., and L.L. were responsible for the collection and assembly of data. P.L., X.H., and X.C. were responsible for data analysis and interpretation. L.X., Z.Q., and F.W. were responsible for manuscript writing. All the authors approved the final manuscript.

Abbreviations

CI, confidence interval; CRC, colorectal cancer; HWE, Hardy–Weinberg equilibrium; MSP, mutagenically separated PCR; MS-PCR, methylation-specific PCR; MTHFR, methylenetetrahydrofolate reductase; OR, odds ratio; PCR-RFLP, PCR-restriction fragment length polymorphism; PCR-SSCP, PCR-single strand conformation polymorphism; PRISMA-P, preferred reporting items for systematic review and meta-analysis protocol; RT-PCR, real-time PCR; SAM, S-adenosylmethionine.

References

1   Siegel, R.L., Miller, K.D. and Jemal, A. (2016) Cancer statistics, 2016. *CA Cancer J. Clin.* **66**, 7–30

2   Markowitz, S.D. and Bertagnolli, M.M. (2009) Molecular basis of colorectal cancer. *N. Engl. J. Med.* **361**, 2449–2460

3   Baroudi, O. and Benammar-elgaaied, A. (2016) Involvement of genetic factors and lifestyle on the occurrence of colorectal and gastric cancer. *Crit. Rev. Oncol. Hemat.* **107**, 72–81

4   Khan, A.A., Khan, Z., Malik, A., Kalam, A.M., Cash, P., Ashraf, T.M. et al. (2017) Colorectal cancer-inflammatory bowel disease nexus and felony of *Escherichia coli*. *Life Sci.* **180**, 60–67

5   Nassiri, M., Kooshyar, M.M., Roudbar, Z., Mahdavi, M. and Doosti, M. (2013) Genes and SNPs associated with non-hereditary and hereditary colorectal cancer. *Asian Pac. J. Cancer Prev.* **14**, 5609–5614

6   Noci, S., Dugo, M., Bertola, F., Melotti, F. and Vannelli, A. (2016) A subset of genetic susceptibility variants for colorectal cancer also has prognostic value. *Pharmacogenomics J.* **16**, 173–179

7   Guo, X.P., Wang, Y., Zhao, H., Song, S.D., Zhou, J. and Han, Y. (2014) Association of *MTHFR* C677T polymorphisms and colorectal cancer risk in Asians: evidence of 12,255 subjects. *Clin. Transl. Oncol.* **16**, 623–629

8   Fang, X., Xu, W., Huang, Q., Yang, X.K., Liu, Y.Y., Leng, R.X. et al. (2014) 5,10-Methylenetetrahydrofolate reductase polymorphisms and colon cancer risk: a meta-analysis. *Asian Pac. J. Cancer Prev.* **15**, 8245–8250

9   Ueland, P.M., Hustad, S., Schneede, J., Refsum, H. and Vollset, S.E. (2001) Biological and clinical implications of the MTHFR C677T polymorphism. *Trends Pharmacol. Sci.* **22**, 195–201

10   Goyette, P., Pai, A., Milos, R., Frosst, P., Tran, P., Chen, Z.T. et al. (1998) Gene structure of human and mouse methylenetetrahydrofolate reductase (*MTHFR*). *Mamm. Genome* **9**, 652–656

11   Rozen, R. (1997) Genetic predisposition to hyperhomocysteinemia: deficiency of methylenetetrahydrofolate reductase (*MTHFR*). *Thromb. Haemost.* **78**, 523–526

12   Frosst, P., Milos, R., Goyette, P., Sheppard, C.A., Matthews, R.G., Boers, G.J.H. et al. (1995) A candidate genetic risk factor for vascular disease:a common mutation in methylenetetrahydrofolate reductase. *Nature* **10**, 111–113

13   Duthie, S.J. (1999) Folic acid deficiency and cancer: mechanisms of DNA instability. *Br. Med. Bull.* **55**, 578–592

14   Zhu, X.L., Liu, Z.Z., Yan, S.X., Wang, W., Chang, R.X., Zhang, Y.C. et al. (2016) Association between the *MTHFR* A1298C polymorphism and risk of cancer: evidence from 265 case-control studies. *Mol. Genet. Genomics* **291**, 51–63

15   Long, S. and Goldblatt, J. (2016) *MTHFR* genetic testing: controversy and clinical implications. *Aust. Fam. Physician* **45**, 237–240

16   Liew, S. and Gupta, E.D. (2015) Methylenetetrahydrofolate reductase (*MTHFR*) C677T polymorphism: Epidemiology, metabolism and the associated diseases. *Eur. J. Med. Genet.* **58**, 1–10

17   Shi, H., Yang, S.W., Liu, Y., Huang, P., Lin, N., Sun, X.R. et al. (2015) Study on environmental causes and SNPs of MTHFR, MS and CBS genes related to congenital heart disease. *PLoS ONE* **10**, e128646

18 Sohda, S., Arinami, T., Hamada, H., Yamada, N. and Hamaguchi, H. (1997) Methylenetetrahydrofolate reductase polymorphism and pre-eclampsia. *J. Med. Genet.* **34**, 525–526

19 Stonek, F. et al. (2007) Methylenetetrahydrofolate reductase C677T polymorphism and pregnancy complications. *Obstet. Gynecol.* **110**, 363–368

20 van der Put, N.M.J., Gabreels, F., Stevens, E.M.B., Smeitink, J.A.M., Trijbels, F.J., Eskes, T.K.A.B. et al. (1998) A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? *Am. J. Hum. Genet.* **62**, 1044–1051

21 Shiao, S.P.K. and Yu, C.H. (2016) Meta-prediction of MTHFR gene polymorphism mutations and associated risk for colorectal cancer. *Biol. Res. Nurs.* **18**, 357–369

22 Shamseer, L., Moher, D., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M. et al. (2015) Preferred reporting items for systematic review and meta-analysis protocols(PRISMA-P)2015:elaboration and explanation. *BMJ* **349**, g7647

23 Wei, G.S. and Thompson, E.A. (1992) Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics* **48**, 361–372

24 Anzures-Cabrera, J. and Higgins, J.P. (2010) Graphical displays for meta-analysis: an overview with suggestions for practice. *Res. Synth. Methods* **1**, 66–80

25 Hayashino, Y., Noguchi, Y. and Fukui, T. (2005) Systematic evaluation and comparison of statistical tests for publication bias. *J. Epidemiol.* **15**, 235–243

26 Haerian, M.S., Haerian, B.S., Molanaei, S., Kosari, F., Sabeti, S., Bidari-Zerepoosh, F. et al. (2016) *MTHFR* rs1801133 polymorphism and susceptibility to colorectal cancer in Iranian population: evidence of a case-control study and meta-analysis. *Pharmacogenomics* **17**, 1957–1965

27 Kim, J.W., Jeon, Y.J., Jang, M.J., Kim, J.O. and Chong, S.Y. (2015) Association between folate metabolism-related polymorphisms and colorectal cancer risk. *Mol. Clin. Oncol.* **3**, 639–648

28 Rai, P.S., Pai, G.C., Alvares, J.F., Bellampalli, R., Gopinath, P.M. and Satyamoorthy, K. (2014) Intraindividual somatic variations in MTHFR gene polymorphisms in relation to colon cancer. *Pharmacogenomics* **15**, 349–359

29 Ozen, F., Sen, M. and Ozdemir, O. (2014) Methylenetetrahydrofolate reductase gene germ-line C677T and A1298C SNPs are associated with colorectal cancer risk in the Turkish population. *Asian Pac. J. Cancer Prev.* **15**, 7731–7735

30 Ashmore, J.H., Lesko, S.M., Muscat, J.E., Gallagher, C.J., Berg, A.S., Miller, P.E. et al. (2013) Association of dietary and supplemental folate intake and polymorphisms in three FOCM pathway genes with colorectal cancer in a population-based case-control study. *Gene. Chromosome Canc.* **52**, 945–953

31 Delgado-Plasencia, L., Medina-Arana, V., Bravo-Gutiérrez, A., Perez-Palma, J., Alvarez-Arguelles, H., Salido-Ruiz, E. et al. (2013) Impact of the *MTHFR* C677T polymorphism on colorectal cancer in a population with low genetic variability. *Int. J. Colorectal Dis.* **28**, 1187–1193

32 Yousef, A., Shomaf, M., Berger, S., Ababneh, N., Bobali, Y., Ali, D. et al. (2013) Allele and genotype frequencies of the polymorphic methylenetetrahydrofolate reductase and colorectal cancer among Jordanian population. *Asian Pac. J. Cancer Prev.* **14**, 4559–4565

33 Lee, J.E., Wei, E.K., Fuchs, C.S., Hunter, D.J., Lee, I.M., Selhub, J. et al. (2012) Plasma folate, methylenetetrahydrofolate reductase (MTHFR), and colorectal cancer risk in three large nested case-control studies. *Cancer Cause Control* **23**, 537–545

34 Promthet, S., Pientong, C., Ekalaksananan, T., Songserm, N., Poomphakwaen, K., Chopjitt, P. et al. (2012) Risk factors for rectal cancer and methylenetetrahydrofolate reductase polymorphisms in a population in northeast Thailand. *Asian Pac. J. Cancer Prev.* **13**, 4017–4023

35 Kim, J., Cho, Y.A., Kim, D.H., Lee, B.H., Hwang, D.Y., Jeong, J. et al. (2012) Dietary intake of folate and alcohol, *MTHFR* C677T polymorphism, and colorectal cancer risk in Korea. *Am. J. Clin. Nutr.* **95**, 405–412

36 Yin, G., Ming, H., Zheng, X., Xuan, Y., Liang, J. and Jin, X. (2012) Methylenetetrahydrofolate reductase C677T gene polymorphism and colorectal cancer risk: a case-control study. *Oncol. Lett.* **4**, 365–369

37 Sameer, A.S., Shah, Z.A., Nissar, S., Mudassar, S. and Siddiqi, M.A. (2011) Risk of colorectal cancer associated with the methylenetetrahydrofolate reductase (*MTHFR*) C677T polymorphism in the Kashmiri population. *Genet. Mol. Res.* **10**, 1200–1210

38 Vossen, C.Y., Hoffmeister, M., Chang-Claude, J.C., Rosendaal, F.R. and Brenner, H. (2011) Clotting factor gene polymorphisms and colorectal cancer risk. *J. Clin. Oncol.* **29**, 1722–1727

39 Kang, B.S., Ahn, D.H., Kim, N.K. and Kim, J.W. (2011) Relationship between metabolic syndrome and *MTHFR* polymorphism in colorectal cancer. *J. Korean Soc. Coloproctol.* **27**, 78–82

40 Zhu, Q., Jin, Z., Yuan, Y., Lu, Q., Ge, D. and Zong, M. (2011) Impact of *MTHFR* gene C677T polymorphism on Bcl-2 gene methylation and protein expression in colorectal cancer. *Scand. J. Gastroenterol.* **46**, 436–445

41 Pardini, B., Kumar, R., Naccarati, A., Prasad, R.B., Forsti, A., Polakova, V. et al. (2011) *MTHFR* and *MTRR* genotype and haplotype analysis and colorectal cancer susceptibility in a case-control study from the Czech Republic. *Mutat. Res.* **721**, 74–80

42 Kim, J.W., Park, H.M., Choi, Y.K., Chong, S.Y. and Oh, D. (2011) Polymorphisms in genes involved in folate metabolism and plasma DNA methylation in colorectal cancer patients. *Oncol. Rep.* **25**, 167–172

43 Prasad, V.V.T.S. and Wilkhoo, H. (2011) Association of the functional polymorphism C677T in the methylenetetrahydrofolate reductase gene with colorectal, thyroid, breast, ovarian, and cervical cancers. *Onkologie* **34**, 422–426

44 Li, H., Xu, W.L., Shen, H.L., Chen, Q.Y., Hui, L.L., Long, L.L. et al. (2011) Methylenetetrahydrofolate reductase genotypes and haplotypes associated with susceptibility to colorectal cancer in an eastern Chinese Han population. *Genet. Mol. Res.* **10**, 3738

45 Jokić, M., Brčić-Kostić, K., Stefulj, J., Ivkovic , T.C., Bozo, L., Gamulin, M. et al. (2011) Association of *MTHFR, MTR, MTRR, RFC1*, and *DHFR* gene polymorphisms with susceptibility to sporadic colon cancer. *DNA Cell Biol.* **30**, 771–776

46 Guimarães, J.L.M., Ayrizono, M.D.L., Coy, C.S.R. and Lima, C.S.P. (2011) Gene polymorphisms involved in folate and methionine metabolism and increased risk of sporadic colorectal adenocarcinoma. *Tumor Biol.* **32**, 853–861

47 Komlósi, V., Hitre, E., Pap, E., Adleff, V., Réti, A., Székely, E. et al. (2010) *SHMT1* 1420 and *MTHFR* 677 variants are associated with rectal but not colon cancer. *BMC Cancer* **10**, 1471–2407

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48 Karpinski, P., Myszka, A., Ramsey, D., Misiak, B., Gil, J., Laczmanska, I. et al. (2010) Polymorphisms in methyl-group metabolism genes and risk of sporadic colorectal cancer with relation to the CpG island methylator phenotype. *Cancer Epidemiol.* **34**, 338–344

49 Cui, L., Shin, M., Kweon, S., Kim, H.N., Song, H., Piao, J. et al. (2010) Methylenetetrahydrofolate reductase C677T polymorphism in patients with gastric and colorectal cancer in a Korean population. *BMC Cancer* **10**, 1471–2407

50 Eussen, S.J.P.M., Vollset, S.E., Igland, J., Meyer, K., Fredriksen, A., Ueland, P.M. et al. (2010) Plasma folate, related genetic variants, and colorectal cancer risk in EPIC. *Cancer Epidemiol. Biomarkers Prev.* **19**, 1328–1340

51 Chandy, S., Adiga, M.N.S., Ramachandra, N., Krishnamoorthy, S., Ramaswamy, G., Savithri, H.S. et al. (2010) Association of methylenetetrahydrofolate reducíase gene polymorphisms & colorectal cancer in India. *Indian J. Med. Res.* **131**, 659–664

52 Naghibalhossaini, F., Mokarram, P., Khalili, I., Vasei, M., Hosseini, S.V., Ashktorab, H. et al. (2010) *MTHFR* C677T and A1298C variant genotypes and the risk of microsatellite instability among Iranian colorectal cancer patients. *Cancer Genet. Cytogenet.* **197**, 142–151

53 Promthet, S.S., Pientong, C., Ekalaksananan, T., Wiangnon, S., Poomphakwaen, K., Songserm, N. et al. (2010) Risk factors for colon cancer in northeastern Thailand: interaction of MTHFR codon 677 and 1298 genotypes with environmental factors. *J. Epidemiol.* **20**, 329–338

54 Yang, X.X., Li, F.X., Yi, J.P., Li, X., Sun, J.Z. and Hu, N.Y. (2010) Impact of methylenetetrahydrofolate reductase C677T polymorphism on the risk of gastric cancer, colorectal cancer and lung cancer. *Guangdong Med.* **31**, 2375–2378

55 Fernández-Peralta, A.M., Daimiel, L., Nejda, N., Iglesias, D., Medina Arana, V. and González-Aguilera, J.J. (2010) Association of polymorphisms *MTHFR* C677T and A1298C with risk of colorectal cancer, genetic and epigenetic characteristic of tumors, and response to chemotherapy. *Int. J. Colorectal Dis.* **25**, 141–151

56 Zhu, F., Wang, Y.-m. and ZhangQY, Q.-y. (2010) A case-control study of plasma homocysteine, serum folate, the polymorphism of methylenetetrahydrofolate reductase in colorectal cancer. *J. Southeast Univ. Med. Sci. Edi.* **29**, 88–92

57 de Vogel, S., Wouters, K.A.D., Gottschalk, R.W.H., van Schooten, F.J., de Goeij, A.F.P.M., de Bruïne, A.P. et al. (2009) Genetic variants of methyl metabolizing enzymes and epigenetic regulators: associations with promoter CpG island hypermethylation in colorectal cancer. *Cancer Epidemiol. Biomarkers Prev.* **18**, 3086–3096

58 Iacopetta, B., Heyworth, J., Girschik, J., Grieu, F., Clayforth, C. and Fritschi, L. (2009) The *MTHFR* C677T and △DNMT3B C-149T polymorphisms confer different risks for right- and left-sided colorectal cancer. *Int. J. Cancer* **125**, 84–90

59 Gallegos-Arreola, M.P., Garcia-Ortiz, J.E., Figuera, L.E., Puebla-Perez, A.M., Morgan-Villela, G., Zuniga-Gonzalez, G.M. et al. (2009) Association of the 677C→T polymorphism in the MTHFR Gene with Colorectal cancer in Mexican patients. *Cancer Genome Proteomics* **6**, 183–188

60 Reeves, S.G., Meldrum, C., Groombridge, C., Spigelman, A.D., Suchy, J., Kurzawski, G. et al. (2009) *MTHFR* 677 C>T and 1298 A>C polymorphisms and the age of onset of colorectal cancer in hereditary nonpolyposis colorectal cancer. *Eur. J. Hum. Genet.* **17**, 629–635

61 El Awady, M.K., Karim, A.M., Hanna, L.S., El Husseiny, L.A., El Sahar, M., Abdel Menem, H.A. et al. (2009) Methylenetetrahydrofolate reductase gene polymorphisms and the risk of colorectal carcinoma in a sample of Egyptian individuals. *Cancer Biomark.* **5**, 233–240

62 Derwinger, K., Wettergren, Y., Odin, E., Carlsson, G. and Gustavsson, B. (2009) A study of the *MTHFR* gene polymorphism C677T in colorectal cancer. *Clin. Colorectal Cancer* **8**, 43–48

63 Haghighi, M.M., Mohebbi, S.R., Khatami, F., Ghiasi, S., Derakhshan, F., Atarian, H. et al. (2008) Reverse association between MTHFR polymorphism (C677T) with sporadic colorectal cancer. *Gastroenterol. Hepatol.* **1**, 57–63

64 Sharp, L., Little, J., Brockton, N.T., Cotton, S.C., Masson, L.F., Haites, N.E. et al. (2008) Polymorphisms in the methylenetetrahydrofolate reductase (*MTHFR*) gene, intakes of folate and related B vitamins and colorectal cancer: a case-control study in a population with relatively low folate intake. *Br. J. Nutr.* **99**, 379–389

65 Kury, S., Buecher, B., Robiou-du-Pont, S., Scoul, C., Colman, H., Neel, T.L. et al. (2008) Low-penetrance alleles predisposing to sporadic colorectal cancers: a French case-controlled genetic association study. *BMC Cancer* **8**, 326

66 Mokarram, P., Naghibalhossaini, F., Firoozi, M.S., Hosseini, S.V., Izadpanah, A., Salahi, H. et al. (2008) Methylenetetrahydrofolate reductase C677T genotype affects promoter methylation of tumor-specific genes in sporadic colorectal cancer through an interaction with folate/vitamin B12 status. *World J. Gastroenterol.* **14**, 3662

67 Cao, H., Gao, C., Takezaki, T., Wu, J., Ding, J., Liu, Y. et al. (2008) Genetic polymorphisms of methylenetetrahydrofolate reductase and susceptibility to colorectal cancer. *Asian Pac. J. Cancer Prev.* **9**, 203–208

68 Theodoratou, E., Farrington, S.M., Tenesa, A., McNeill, G., Cetnarskyj, R., Barnetson, R.A. et al. (2008) Dietary vitamin B6 intake and the risk of colorectal cancer. *Cancer Epidemiol. Biomarkers Prev.* **17**, 171–182

69 Eklöf, V., Van Guelpen, B., Hultdin, J., Johansson, I., Hallmans, G. and Palmqvist, R. (2009) The reduced folate carrier (*RFC1*) 80G>A and folate hydrolase 1 (*FOLH1*) 1561C>T polymorphisms and the risk of colorectal cancer: a nested case-referent study. *Scand. J. Clin. Lab. Inv.* **68**, 393–401

70 Zhang, Y.L., Yuan, X.Y., Zhang, C., Yang, Y., Pan, Y.M., Zhou, Z.Y. et al. (2008) Relationship between polymorphisms of thymidylate synthase and methylenetetrahydrofolate reductase and susceptibility in Liaoning Benxi colorectal cancer patients. *Cancer J. Clin.* **13**, 769–773

71 Guerreiro, C.S., Carmona, B., Gonçalves, S., Carolino, E., Fidalgo, P., Brito, M. et al. (2008) Risk of colorectal cancer associated with the C677T polymorphism in 5,10-methylenetetrahydrofolate reductase in Portuguese patients depends on the intake of methyl-donor nutrients. *Am. J. Clin. Nutr.* **88**, 1413–1418

72 Osian, G., Procopciuc, L. and Vlad, L. (2007) *MTHFR* polymorphisms as prognostic factors in sporadic colorectal cancer. *J. Gastrointestin. Liver Dis.* **16**, 251–256

73 Zeybek, U., Yaylim, I., Yilmaz, H., Agachan, B., Ergen, A., Arikan, S. et al. (2007) Methylenetetrahydrofolate reductase C677T polymorphism in patients with gastric and colorectal cancer. *Cell Biochem. Funct.* **25**, 419–422

74 Lima, C.S.P., Nascimento, H., Bonadia, L.C., Teori, M.T., Coy, C.S.R., Goes, J.R.N. et al. (2007) Polymorphisms in methylenetetrahydrofolate reductase gene (*MTHFR*) and the age of onset of sporadic colorectal adenocarcinoma. *Int. J. Colorectal Dis.* **22**, 757–763

75 Chang, S., Lin, P., Lin, J., Yang, S., Wang, H. and Li, A. (2007) Role of *MTHFR* polymorphisms and folate levels in different phenotypes of sporadic colorectal cancers. *Int. J. Colorectal Dis.* **22**, 483–489

76 Murtaugh, M.A., Curtin, K., Sweeney, C., Wolff, R.K., Holubkov, R. and Caan, B.J. (2007) Dietary intake of folate and co-factors in folate metabolism, MTHFR polymorphisms, and reduced rectal cancer. *Cancer Cause Control* **18**, 153–163

77 Jin, X.X., Zhu, Z.Z., Wang, A.Z. and Jia, H.R. (2007) Association of methylenetetrahydrofolate reductase C677T polymorphism with genetic susceptibility to colorectal cancer. *World Chin. J. Dig.* **15**, 2754–2757

78 Curtin, K., Slattery, M.L., Ulrich, C.M., Bigler, J., Levin, T.R., Wolff, R.K. et al. (2007) Genetic polymorphisms in one-carbon metabolism: associations with CpG island methylator phenotype (CIMP) in colon cancer and the modifying effects of diet. *Carcinogenesis* **28**, 1672–1679

79 Hubner, R.A., Lubbe, S., Chandler, I. and Houlston, R.S. (2007) MTHFR C677T has differential influence on risk of MSI and MSS colorectal cancer. *Hum. Mol. Genet.* **16**, 1072–1077

80 Koushik, A., Kraft, P., Fuchs, C.S., Hankinson, S.E., Willett, W.C., Giovannucci, E.L. et al. (2006) Nonsynonymous polymorphisms in genes in the one-carbon metabolism pathway and associations with colorectal cancer. *Cancer Epidemiol. Biomarkers Prev.* **15**, 2408–2417

81 Battistelli, S., Vittoria, A., Stefanoni, M., Bing, C. and Roviello, F. (2006) Total plasma homocysteine and methylenetetrahydrofolate reductase C677T polymorphism in patients with colorectal carcinoma. *World J. Gastroenterol.* **12**, 6128–6132

82 Van Guelpen, B., Hultdin, J., Johansson, I., Hallmans, G., Stenling, R., Riboli, E. et al. (2006) Low folate levels may protect against colorectal cancer. *Gut* **55**, 1461–1466

83 Wang, J., Gajalakshmi, V., Jiang, J., Kuriki, K., Suzuki, S., Nagaya, T. et al. (2006) Associations between 5,10-methylenetetrahydrofolate reductase codon 677 and 1298 genetic polymorphisms and environmental factors with reference to susceptibility to colorectal cancer: a case-control study in an Indian population. *Int. J. Cancer* **118**, 991–997

84 Chen, K., Song, L., Jin, M.J., Fang, C.H., Jiang, X.D. and Yu, W.P. (2006) Associations between folate metabolism enzyme gene polymorphisms and colorectal susceptibility. *Chin. J. Oncol.* **28**, 429–432

85 Matsuo, K., Ito, H., Wakai, K., Hirose, K., Saito, T., Suzuki, T. et al. (2005) One-carbon metabolism related gene polymorphisms interact with alcohol drinking to influence the risk of colorectal cancer in Japan. *Carcinogenesis* **26**, 2164–2171

86 Landi, S., Gemignani, F., Moreno, V., Gioia-Patricola, L., Chabrier, A., Guino, E. et al. (2005) A comprehensive analysis of phase I and phase II metabolism gene polymorphisms and risk of colorectal cancer. *Pharmacogenet. Genomics* **15**, 535–546

87 Le Marchand, L., Wilkens, L.R., Kolonel, L.N. and Henderson, B.E. (2005) The *MTHFR* C677T polymorphism and colorectal cancer: the multiethnic cohort study. *Cancer Epidemiol. Biomarkers Prev.* **14**, 1198–1203

88 Jiang, Q., Chen, K., Ma, X.Y., Yao, K.Y., Yu, W.P., Li, L.Y. et al. (2005) Diets, polymorphisms of methylenetetrahydrofolate reductase, and the susceptibility of colon cancer and rectal cancer. *Cancer Detect. Prev.* **29**, 146–154

89 Otani, T., Iwasaki, M., Hanaoka, T., Kobayashi, M., Ishihara, J., Natsukawa, S. et al. (2005) Folate, vitamin B6, vitamin B12, and vitamin B2 intake, genetic polymorphisms of related enzymes, and risk of colorectal cancer in a hospital-based case-control study in Japan. *Nutr. Cancer* **53**, 42–50

90 Miao, X.P., Yang, S., Tan, W., Zhang, X.M., Ye, Y.J., Lin, Y.J. et al. (2005) Association between genetic variations in methylenetetrahydrofolate reductase and risk of colorectal cancer in a Chinese population. *Chin. Prev. Med.* **39**, 409–411

91 Kim, D., Ahn, Y., Lee, B., Tsuji, E., Kiyohara, C. and Kono, S. (2004) Methylenetetrahydrofolate reductase polymorphism, alcohol intake, and risks of colon and rectal cancers in Korea. *Cancer Lett.* **216**, 199–205

92 Ulvik, A., Vollset, S.E., Hansen, S., Gislefoss, R., Jellum, E. and Ueland, P.M. (2004) Colorectal cancer and the methylenetetrahydrofolate reductase 677C→T and methionine synthase 2756A→G polymorphisms: a study of 2,168 case-control pairs from the JANUS cohort. *Cancer Epidemiol. Biomarkers Prev.* **13**, 2175–2180

93 Yin, G., Kono, S., Toyomura, K., Hagiwara, T., Nagano, J., Mizoue, T. et al. (2004) Methylenetetrahydrofolate reductase C677T and A1298C polymorphisms and colorectal cancer: the Fukuoka Colorectal Cancer Study. *Cancer Sci.* **95**, 908–913

94 Curtin, K., Bigler, J., Slattery, M.L., Caan, B., Potter, J.D. and Ulrich, C.M. (2003) *MTHFR* C677T and A1298C polymorphisms: diet, estrogen, and risk of colon cancer. *Cancer Epidemiol. Biomarkers Prev.* **13**, 285–292

95 Pufulete, M., Al-Ghnaniem, R., Leather, A.J.M., Appleby, P., Gout, S., Terry, C. et al. (2003) Folate status, genomic DNA hypomethylation, and risk of colorectal adenoma and cancer: a case control study. *Gastroenterology* **124**, 1240–1248

96 Plaschke, J., Schwanebeck, U., Pistorius, S., Saeger, H.D. and Schackert, H.K. (2003) Methylenetetrahydrofolate reductase polymorphisms and risk of sporadic and hereditary colorectal cancer with or without microsatellite instability. *Cancer Lett.* **191**, 179–185

97 Toffoli, G., Gafà, R., Russo, A., Lanza, G., Dolcetti, R., Sartor, F. et al. (2003) Methylenetetrahydrofolate reductase 677 C → T polymorphism and risk of proximal colon cancer in north Italy. *Clin. Cancer Res.* **9**, 743–748

98 Heijmans, B.T., Boer, J.M.A., Suchiman, H.E.D., Cornelisse, C.J., Westendorp, R.G.J., Kromhout, D. et al. (2003) A common variant of the methylenetetrahydrofolate reductase gene (1p36) is associated with an increased risk of cancer. *Cancer Res.* **63**, 1249–1253

99 Huang, P., Zhou, Z.Y., Ma, H.T., Liu, J.Y., Zhou, Y.H., Cao, J. et al. (2003) *MTHFR* polymorphisms and colorectal cancer susceptibility in Chongqing people. *Acta Acad. Med. Mil. Tert.* **25**, 1704–1710

100 Barna, B., Erika, H., Vilmos, A., Ferenc, C., Fruzsina, G., Istvan, L. et al. (2004) A metiléntetrahidrofolát-reduktáz (*MTHFR*) C677T polimorfizmus klinikai jelentôsége a metasztatikus colorectalis daganatok 5-fluoropirimidin-alapú kezelésében. *Magyar Onkológia* **48**, 253–257

101 Keku, T., Millikan, R., Worley, K., Winkel, S., Eaton, A., Biscocho, L. et al. (2002) 5,10-Methylenetetrahydrofolate reductase codon 677 and 1298 polymorphisms and colon cancer in African and Whites. *Cancer Epidemiol. Biomarkers Prev.* **11**, 1611–1621

102 Le Marchand, L., Donlon, T., Hankin, J.H., Kolonel, L.N., Wilkens, L.R. and Seifried, A. (2002) B-vitamin intake, metabolic genes, and colorectal cancer risk (United States). *Cancer Cause Control* **13**, 239–248

103 Shannon, B., Gnanasampanthan, S., Beilby, J. and Iacopetta, B. (2002) A polymorphism in the methylenetetrahydrofolate reductase gene predisposes to colorectal cancers with microsatellite instability. *Gut* **50**, 520–524

104 Matsuo, K., Hamajima, N., Hirai, T., Kato, T. and Inoue, M. (2002) Methionine synthase reductase gene A66G polymorphism is associated with risk of colorectal cancer. *Asian Pac. J. Cancer Prev.* **3**, 353–359

105 Sachse, C., Smith, G., Wilkie, M., Barrett, J.H. and Waxman, R. (2002) A pharmacogenetic study to investigate the role of dietary carcinogens in the etiology of colorectal cancer. *Carcinogenesis* **23**, 1839–1849

106 Chen, J., Ma, J., Stampfer, M.J., Palomeque, C., Selhub, J. and Hunter, D.J. (2002) Linkage disequilibrium between the 677C>T and 1298A>C polymorphisms in human methylenetetrahydrofolate reductase gene and their contributions to risk of colorectal cancer. *Pharmacogenetics* **12**, 339–342

107 Ryan, B.M., Molloy, A.M., McManus, R., Arfin, Q., Kelleher, D., Scott, J.M. et al. (2001) The methylenetetrahydrofolate reductase (MTHFR) gene in colorectal cancer. *Int. J. Gastrointestin. Cancer* **30**, 105–111

108 Slattery, M.L., Edwards, S.L., Samowitz, W. and Potter, J. (2000) Associations between family history of cancer and genes coding for metabolizing enzymes (United States). *Cancer Cause Control* **11**, 799–803

109 Slattery, M.L., Potter, J.D. and Samowitz, W. (1999) Methylenetetrahydrofolate reductase, diet, and risk of colon cancer. *Cancer Epidemiol. Biomarkers Prev.* **8**, 513–518

110 Park, K.S., Mok, J.W. and Kim, J.C. (1999) The 677C >T mutation in 5,10-methylenetetrahydrofolate reducíase and colorectal cancer risk. *Genet. Test* **3**, 233–236

111 Jing, M., Stampfer, M.J. and Giovannucci, E. (1997) Methylenetetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer. *Cancer Res.* **57**, 1098–1102

112 Chen, J., Giovannucci, E. and Kelsey, K. (1996) A methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer. *Cancer Res.* **56**, 4862–4864

113 Zhou, D., Mei, Q., Luo, H., Tang, B. and Yu, P. (2012) The polymorphisms in methylenetetrahydrofolate reductase, methionine synthase, methionine synthase reductase, and the risk of colorectal cancer. *Int. J. Biol. Sci.* **8**, 819–830

114 Kennedy, D.A., Stern, S.J., Matok, I., Moretti, M.E., Sarker, M., Adams-Webber, T. et al. (2012) Folate intake, *MTHFR* polymorphisms, and the risk of colorectal cancer: a systematic review and meta-analysis. *J. Cancer Epidemiol.* **2012**, 952508

115 Haerian, B.S. and Haerian, M.S. (2015) Evaluation of association studies and meta-analyses of *MTHFR* gene polymorphisms in colorectal cancer. *Pharmacogenomics* **16**, 413–425

116 Yang, Z., Zhang, X., Liu, H., Hao, Y. and Zhao, C. (2012) *MTHFR* C677T polymorphism and colorectal cancer risk in asians, a meta-analysis of 21 Studies. *Asian Pac. J. Cancer Prev.* **13**, 1203–1208

117 Guo, X.P., Wang, Y., Zhao, H., Song, S.D., Zhou, J. and Han, Y. (2014) Association of *MTHFR* C677T polymorphisms and colorectal cancer risk in Asians: evidence of 12,255 subjects. *Clin. Transl. Oncol.* **16**, 623–629

118 Zhong, S., Yang, J., Liu, K., Jiao, B.H. and Chang, Z. (2012) Quantitative assessment of the association between *MTHFR* C677T polymorphism and colorectal cancer risk in East Asians. *Tumor Biol.* **33**, 2041–2051