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ORIGINAL ARTICLE

Different roles of *MTHFR* C677T and A1298C polymorphisms in colorectal adenoma and colorectal cancer: a meta-analysis

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Abstract Association studies on the *MTHFR* polymorphisms (C677T and A1298C) in colorectal cancer (CRC) and colorectal adenoma have shown conflicting results. We performed a meta-analysis to better assess the purported associations. Overall, the 677T allele (10,131 patients and 15,362 controls) showed a small but significant protective effect against CRC compared to the 677C allele [P=0.0003, odds ratio (OR)=0.93; 95% confidence interval (CI) 0.89–0.98, P=0.22 (for heterogeneity)] for a worldwide population. Meta-analyses of other genetic contrasts suggested that the 677T allele is more likely to affect CRC in a recessive genetic model worldwide (P<0.0001, OR=0.86; 95% CI 0.76–0.96, P=0.06) and in Asians (P=0.0005, OR=0.75; 95% CI 0.64–0.88, P=0.71). Similarly, we found a significantly decreased risk of CRC for 1298C polymorphism (4,764 CRC patients and 6,592 controls) for a recessive genetic model worldwide (P=0.005, OR=0.81; 95% CI 0.70–0.94, P=0.40) and in Caucasians (P=0.04, OR=0.75 95% CI 0.57–0.99, P=0.35). No evidence of association of C677T (4,616 patients and 6,338 controls) and A1298C (1,272 patients and 1,684 controls) with colorectal adenoma were found. The evidence accumulated suggests that *MTHFR* may represent a low-penetrance susceptible gene for CRC, and that the two polymorphisms might protect against colorectal adenoma developing into cancer. A larger single study is required to further evaluate gene–gene and gene–environment interactions for *MTHFR* polymorphisms and the cancer risk in a specific population.

Keywords MTHFR · Colorectal cancer · Colorectal adenoma · Polymorphism · Meta-analysis

Introduction

Colorectal cancer (CRC) is one of the most common cancers, and the development of CRC is determined by a series of risk factors, such as genetic polymorphisms, environmental factors, lifestyles, and the complex interaction between these factors (de Kok and van Maanen 2000). Colorectal adenomas are well-established precursor lesions for colorectal cancer (Fearon and Vogelstein 1990; Peipins and Sandler 1994); prevention of colorectal adenomas may decrease the occurrence of colorectal cancer.

It is widely accepted that gene methylation is implicated in carcinogenesis (Toyota et al. 1999). 5,10-Methylenetetrahydrofolate reductase (MTHFR) is involved in DNA methylation; it manipulates the levels of folate, one of the methyl group donors (Kim et al. 1996). MTHFR catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate (required for purine and thymidine synthesis) to 5-methyltetrahydrofolate, which is necessary for methionine synthesis (Ueland et al. 2001). Insufficient thymidylate results in uracil misincorporation into DNA, leading to single-strand and double-strand breaks and increasing the incidence

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of DNA misrepair, thus increasing the risk of genetic instability (Blount et al. 1997).

MTHFR C677T (Ala222Val) (Frosst et al. 1995) and A1298C (Glu429Ala) (van der Put et al. 1998) are two common polymorphisms. Both of these polymorphisms decrease the activity of the enzyme; this effect is more pronounced in the homozygous than the heterozygous state (Ogino and Wilson 2003). The *MTHFR* C677T polymorphism exerts opposite effects in colorectal carcinogenesis, depending on the balance of thymidylate synthesis and DNA methylation, which affects the folate pool (Giovannucci et al. 2003). An association between *MTHFR* and genetic susceptibility to CRC and colorectal adenoma has been widely documented but with inconsistent results. A single study may be underpowered to detect a possible small effect from a real association of these polymorphisms with CRC and colorectal adenoma, especially when the sample size is relatively small. Different populations and sampling methods were applied in each study, which makes it complicated to interpret the data. Thus, a rigorous quantitative synthesis method is required in order to assess the discrepancy.

Here, we performed a meta-analysis of all eligible case-control studies in order to address the association of *MTHFR* C677T and A1298C polymorphisms with CRC and colorectal adenoma. Our results indicated that both the 677T and 1298C alleles might have a protective effect from CRC across a worldwide population. The 677T allele may exert its effect on CRC in a recessive genetic model in Asians, while the 1298C allele may decrease CRC risk in a recessive genetic model in Caucasians. No associations of C677T and A1298C polymorphisms were found to increase the risk of colorectal adenoma, which indicated that the two polymorphisms may protect from the adenoma becoming malignant cancer. More studies are needed to further confirm the protective roles of these polymorphisms in CRC development, and their effects in different populations.

Materials and methods

Identification and eligibility of relevant studies

To identify all articles that examined the association of *MTHFR* C677T and A1298C polymorphisms with CRC and colorectal adenoma, we conducted a literature search of the PubMed database (from January 1991 to August 2006) using the following keywords and subject terms: ‘‘MTHFR,’’ ‘‘polymorphism,’’ and ‘‘colorectal cancer’’ or ‘‘colorectal adenoma.’’ ‘‘Colon cancer (adenoma)’’ and ‘‘rectal cancer (adenoma)’’ were used to replace ‘‘colorectal cancer (adenoma)’’ in further searches of related studies. References in retrieved articles were screened. Abstracts, case reports, editorials, and review articles were excluded. Studies included in the current meta-analysis had to meet all of the following criteria: (a) an unrelated case-control design was used, (b) genotype frequency was available, and (c) the genotype distribution of the control population must be in Hardy–Weinberg equilibrium (HWE).

Data extraction

Data were collected on the genotypes of C677T or A1298C based on colorectal cancer and colorectal adenoma, respectively. First author, year of publication, ethnicity of study population, and characteristics of cases and controls were described.

Statistical analysis

The meta-analysis examined the overall association of the C677T allele T with the risk of CRC and colorectal adenoma compared to that for allele C; homozygote TT was contrasted with CC, recessive (TT vs. CT + CC) and dominant (TT + CT vs. CC) models for allele T. The same contrasts were performed for allele C of the A1298C polymorphism.

Odds ratios (OR) corresponding to a 95% confidence interval (CI) were applied to assess the strength of association of C677T or A1298C with CRC and colorectal adenoma since case-control studies were used, and OR was calculated according to the method of Woolf (1955). A chi-square-based Q statistic test was performed to assess the between-study heterogeneity (Lau et al. 1997). Heterogeneity was considered significant for $P<0.10$ because of the low power of the statistic. A fixed-effect model using the Mantel–Haenszel method and a random-effects model using the DerSimonian and Laird method were used to pool the results (Petitti 1994). In the absence of between-study heterogeneity, the two methods provide similar results. Random effects are more appropriate when heterogeneity is present. The significance of the pooled OR was determined by the Z test, a P value of <0.05 was considered significant. For each genetic contrast, subgroup analysis according to ethnicity was only considered for Asian and Caucasian populations, in order to estimate ethnic-specific OR. The A1298C comparisons for colorectal adenoma were not stratified for subgroup analysis because of the limited studies included.

Publication bias was investigated by funnel plot, in which the standard error in log(OR) in each study was plotted against OR. An asymmetric plot suggested possible publication bias. Funnel plot asymmetry was assessed via Egger's linear regression test, a linear regression approach to measuring funnel plot asymmetry on the natural logarithm scale of the OR (Egger et al. 1997). The significance of the intercept was determined by the *t*-test as suggested by Egger, and a *P* value of <0.05 was considered significant.

Hardy–Weinberg equilibrium was tested for by the chi-square test for goodness of fit using a web-based program (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). Analyses were performed using the software Stata version 7, ReviewManage 4.2. All *P* values were two-sided.

Results

Selection of studies

Twenty-eight articles were retrieved based on the search criteria for colorectal cancer, and 20 met our inclusion criteria. For various reasons, eight articles were not included. Genotype distributions in the control populations in five studies (Le Marchand et al. 2005; Jiang et al. 2005; Ma et al. 1997; Chen et al. 2002; Miao et al. 2005) significantly deviated from HWE. No detailed genotyping information was available from Marugame et al. (2003) and Kawakami et al. (2003). One article (Slattery et al. 1997) was replaced with its updated study (Slattery et al. 1999). All 20 eligible articles described C677T (Chen et al. 1996; Park et al. 1999; Slattery et al. 1999; Ryan et al. 2001; Keku et al. 2002; Le Marchand et al. 2002; Sachse et al. 2002; Shannon et al. 2002; Heijmans et al. 2003; Plaschke et al. 2003; Pufulete et al. 2003; Toffoli et al. 2003; Curtin et al. 2004; Jiang et al. 2004; Kim et al. 2004; Ulvik et al. 2004; Yin et al. 2004; Matsuo et al. 2005; Otani et al. 2005; Wang et al. 2006), and 11 of them also provide data on A1298C (Table 1). Among the 20 eligible articles included, 70% (14/20) stated that the age and sex status were matched between the case and control populations. PCR-RFLP was used to validate genotype in all but two articles. Ulvik et al. (2004) used real-time PCR and Otani et al. (2005) used Taqman SNP genotyping assay. All of the articles used blood samples for genotyping except Shannon et al. (2002), which used frozen tissue samples.

Thirteen articles were retrieved for colorectal adenoma. Crabtree et al. (2004) only examined the *MTHFR* polymorphism with respect to the severity of the adenoma. Ulvik et al. (2001) contained no detailed information on genotyping. These two studies were discarded. Among 11 eligible articles (Chen et al. 1998; Ulrich et al. 1999; Levine et al. 2000; Marugame et al. 2000; Giovannucci et al. 2003; Pufulete et al. 2003; Boyapati et al. 2004; Goode et al. 2004; van den Donk et al. 2005; Hirose et al. 2005; Mitrou et al. 2006), 27% (3/11) stated that the age and sex status were matched between the case and control populations. PCR-RFLP was used to validate the genotype in all of the studies. All articles included in the meta-analysis provided information on C677T, and three of them also genotyped A1298C (Table 1).

Keku et al. (2002) provided data on two ethnicities (African–Americans and Caucasians), while Le Marchand et al. (2002) reported on three separate populations: Japanese, Caucasians, Hawaiians. Thus, each subpopulation in these two articles was treated as a separate study in the meta-analysis. Studies that provide genotyping data for mixed populations are indicated as having ''unknown'' ethnicity in Table 1.

Summary statistics

A total of 10,131 CRC patients and 15,362 controls (C677T), 4,764 CRC patients and 6,592 controls (A1298C), 4,616 adenoma patients and 6,338 controls (C677T), and 1,272 adenoma patients and 1,684 controls (A1298C) were investigated. Electronic Supplementary Material Table 1 shows the genotype and allele frequencies of both CRC and colorectal adenoma patients as well as the controls in the eligible studies. The allele frequencies were calculated for controls from the corresponding genotype distributions. The variant allele frequencies of 677T and 1298C in the individual studies are also shown in Table 1.

The C677 allele was a little less common among controls of Asian descent (62.4%, 95% CI 54.0–70.8) than in controls of European descent (67.8%, 95% CI 65.1–70.5). The A1298 allele was slightly more common in controls of Asian descent (77.7%, 95% CI 68.0–87.3) than in controls of European descent (71.0%, 95% CI 65.8–76.2). Overall, the prevalence of 677TT homozygosity was 15.6% and 16.8% in control subjects of Asian and European descent, respectively. The prevalence of 1298CC homozygosity was 5.3 and 9.0% in control subjects of Asian and European descent, respectively.

Genetic contrasts

No significant heterogeneity existed between the 23 studies upon comparing the C677T C to the T allele in CRC (P=0.22). First, a fixed-effect model was used to

Table 1 Characteristics of eligible studies considered in the meta-analysis

First author (year)	Ethnicity	SNP studied and variant allele frequency (%)	Case	Control
Studies on colorectal cancer				
Wang (2006)	Asian	C677T(6.2) A1298C(40.7)	302 subjects first diagnosed with colorectal carcinoma, aged from 17 to 75, median age 50, mean age 47.3 (±12.6)	291 age- and sex-matched cancer-free healthy controls from relatives or visitors, age from 20 to 75, median age 50, mean age 48.5 (±12.0)
Matsuo (2005)	Asian	C677T(39.9) A1298C(20.8)	257 patients histologically diagnosed as having CRCs, mean age 58.8 (±10.3)	771 age- and sex-matched first-visit outpatients from the same hospital, mean age 59.0 (±10.2)
Otani (2005)	Asian	C677T(51.3) A1298C(16.3)	106 patients newly diagnosed with histologically confirmed colorectal cancer, age 20–74, mean age 60 (±9)	222 healthy controls matched with cases by sex, age, and residential area, mean age 60 (±9)
Curtin (2004)	Unknown	C677T(33.3) A1298C(31.9)	1,608 subjects diagnosed with first-primary incident colon cancer, age 30–79, mean age 64.4 (±9.8)	1,972 age- and sex-matched controls with no previous colorectal tumor, age 30–79, mean age 64.5 (±10.1)
Jiang (2004)	Asian	C677T(39.7) A1298C(17.1)	125 subjects with adenocarcinoma in colon or rectum, aged 35–80	340 age- and sex-matched healthy controls from the same county, aged 38–89
Kim (2004)	Asian	C677T(38.9)	243 patients histologically confirmed as having incident colorectal adenocarcinomas, age 30–79, mean age 56 (±11)	225 age- and sex-matched controls selected from patients with a wide spectrum of non-neoplastic conditions, aged 30–79 years, mean age 55 (±11)
Ulvik (2004)	Asian	C677T(29.9)	2159 subjects histologically diagnosed as adenocarcinoma in colon or rectum, age from 20 to 67	2190 randomly selected controls from the same county matched by age (±6 months) and sex
Yin (2004)	Unknown	C677T(40.7) A1298C(18.1)	685 histologically confirmed incident colorectal adenocarcinomas, age 20–74	788 community controls selected randomly in the study area as for the cases, age 20–74, with allowance for proportions of residents by sex and ten-year age class
Heijmans (2003)	Caucasian	C677T(28.9)	18 men aged 65–84 who were followed for >10 years in Zutphen Elderly Study with CRC verified by hospital discharge data	793 men aged 65–84 who were followed for >10 years in the Zutphen Elderly Study
Plaschke (2003)	Caucasian	C677T(34.0) A1298C(33.7)	287 patients with sporadic primary colorectal cancer and 60 patients with hereditary nonpolyposis colorectal cancer	346 healthy controls randomly selected from the same region as the cases
Pufulete (2003)	Caucasian	C677T(27.0) A1298C(21.1)	28 individuals with histologically confirmed colorectal cancer, mean age 68.9 (±2.2)	76 control patients with no previous or current history of colorectal polyps or cancer on full colonoscopy, mean age 58.0 (±12.9)
Toffoli (2003)	Caucasian	C677T(45.2) A1298C(30.6)	276 patients with histopathological confirmed cancer in the proximal or distal colon, age from 23 to 91, median age 68	279 control individuals from the same geographic area as the cases, age from 19 to 66, median age 37
Keku (2002)	African, Caucasian	C677T(C30.1)[a] A1298C(C34.4)[a]	552 people with a first diagnosis of histologically confirmed invasive adenocarcinoma of the colon, age between 40 and 79, median age 65	868 healthy controls were frequency-matched to cases by race, sex, and by five-year age group, median age 66.5

Table 1 continued

First author (year)	Ethnicity	SNP studied and variant allele frequency (%)	Case	Control
Le Marchand et al. (2002)	Asian, Caucasian, Hawaiian	C677T(A42.3,C37.7)[a] A1298C(A21.0,C30.7)[a]	548 subjects diagnosed with a primary adenocarcinoma of the colon or rectum, median age 66 (57–73)	656 controls were selected from participants in a health survey, One control was matched to each case by sex, ethnicity, and age (±2 years). Median age 67 (58–74)
Sachse (2002)	Caucasian	C677T(31.3)	490 patients with CRC (45–80 years), mean age 67.70 (±8.50)	592 healthy population-based controls, mean age 68.61 (±8.89), with 433 age- and sex-matched to patients (case–control pairs)
Shannon (2002)	Caucasian	C677T(32.6)	501 frozen or routinely processed tumor samples obtained from 501 patients confirmed with CRC, median age 70	1,207 age- and sex-matched healthy individuals, aged 20–92 years
Ryan (2001)	Caucasian	C677T(29.0)	136 unselected cases of sporadic CRC, median age 68	848 normal population controls
Park (1999)	Asian	C677T(42.8)	200 colorectal cancer patients at different stages	460 healthy, unrelated Korean adults without colorectal cancer
Slattery (1999)	Unknown	C677T(33.0)	1,467 subjects diagnosed with primary colon cancer, age 30–79	1,821 controls were matched to cases by five-year age groups and sex
Chen (1996)	Unknown	C677T(34.3)	144 men diagnosed with colorectal cancer between 1980 and 1994 from a prospective HPFS (male health professionals) study, age 40–75	627 cancer-free men from the same study as the cases, age 40–75
Studies for colorectal adenoma				
Mitrou (2006)	Caucasian	C677T(32.6) A1298C(31.5)	868 cases with adenoma, age 55–64, mean age 60.8 (±2.9)	898 polyp-free participants age- and sex-matched to the cases, aged 55–66 years, mean age 60.7 (±2.9)
Donk et al. (2005)	Caucasian	C677T(32.7)	768 subjects with at least one histologically confirmed colorectal adenoma in their lifetimes, mean age 59.1 (±10.1)	709 controls with no history of any type of polyp, age 18–75 years, mean age 51.5 (±13.6)
Hirose (2005)	Asian	C677T(38.4)	452 cases of histologically confirmed colorectal adenoma, all male officials in the Self-Defense Forces, examined between 1997 and 2001	1,050 controls with no polyp who underwent total colonoscopy in the same period, male officials from the same Self-Defense Forces as the cases
Boyapati (2004)	Unknown	C677T(28.1)	177 patients with concurrent adenomas and hyperplastic polyps, age 30–74, mean age 58.4 (±8.4)	228 healthy individuals and patients who had only hyperplastic polyps, age 30–74, mean age 56.0 (±10.0)
Goode (2004)	Caucasian	C677T(33.0)	481 cases with histologically confirmed adenoma, age 30–74	564 polyp-free participants without inflammatory bowel disease, aged 30–74 years
Giovannucci (2003)	Unknown	C677T(36.3) A1298C(28.5)	374 cases with histologically confirmed adenoma from a prospective study on male physician health (1986–1995), aged 45–70 years	725 participants free of colorectal polyps from the same prospective study on male physician health, aged 45–70 years
Pufulete (2003)	Caucasian	C677T(27.0) A1298C(21.1)	35 individuals with histologically confirmed colorectal adenoma, mean age 66.4 (±12.4)	76 control patients with no previous or current history of colorectal polyps or cancer on full colonoscopy, mean age 58.0 (±12.9)

Table 1 continued

First author (year)	Ethnicity	SNP studied and variant allele frequency (%)	Case	Control
Levine (2000)	Unknown	C677T(29.0)	471 subjects with histologically confirmed adenomas, age 50–74	510 age-, sex- and race-matched controls with no adenomas of any type who took sigmoidoscopy in the same hospitals as the cases
Marugame (2000)	Asian	C677T(35.7)	205 cases of histologically confirmed colorectal adenoma, all male officials in the Self-Defense Forces, examined from 1995 to 1996, age 47–55	220 controls with no polyp who underwent total colonoscopy in the same period, male officials from the same Self-Defense Forces as the cases, age 47–55
Ulrich (1999)	Unknown	C677T(32.0)	527 patients diagnosed with colonoscopically confirmed colon or rectal adenomatous polyps, ages 30–74 years, mean age 58.1 (±9.7)	645 controls derived from the same gastroenterology practice that were polyp-free at colonoscopy, age 30–74, mean age 52.8 (±10.9)
Chen (1998)	Unknown	C677T(32.0)	258 cases of first incident distal or proximal colorectal adenoma, aged 30–55, all female	713 matched controls with no colorectal adenomas, age 30–55, all female

Some of the studies were marked as "unknown" ethnicity because the genotyping data was from a mixture of different populations

A Asians, *C* Caucasians

[a] The variant allele frequency is only shown for populations stratified for subgroup analysis

pool the results (Fig. 1A). There was evidence that the T allele resulted in decreased susceptibility to CRC in a worldwide population. The overall OR was 0.93, 95% CI (0.89–0.98) by fixed effects (P=0.004). No significant between-study heterogeneity was found within each subgroup. The decreased effect of the T allele on the susceptibility was seen in both subgroups, with eight comparisons in populations of Asian descent (P=0.001, OR=0.87, 95% CI 0.81–0.95) and nine comparisons in those of Caucasian descent (P=0.03, OR=0.93, 95% CI 0.88–0.99). Meta-analyses for other genetic contrasts further suggested that the 677T allele was more likely to affect the CRC risk in a recessive genetic model worldwide (P=0.009, OR=0.86, 95% CI 0.76–0.96, random effects model), with between-study heterogeneity present (P=0.06). This result implies that a dose effect may exist for this polymorphism. Interestingly, this effect is only significant in Asians (P=0.0005, OR=0.75, 95% CI 0.64–0.88, P=0.71 for heterogeneity), not in Caucasians (P=0.16, OR=1.02, 95% CI 0.79–1.32, P=0.006 for heterogeneity). The TT versus CC contrast also showed that the association between C677T polymorphism and CRC risk was maintained in Asians but not Caucasians (Table 2).

Eleven studies investigated the association between C677T polymorphism and colorectal adenoma. No heterogeneity was found among the studies (P=0.87) when comparing the C to the T allele. No association was found with colorectal adenoma when a fixed model was used to perform a worldwide allele comparison (Fig. 1B, P=0.52, OR=0.98, 95% CI 0.93–1.04), as well as comparisons for Asian (P=0.78, OR=0.98, 95% CI 0.85–1.13) and Caucasian (P=0.35, OR=0.96, 95% CI 0.88–1.05) populations. No evidence of association between C677T and colorectal adenoma was discerned upon comparing with other genetic models.

There was heterogeneity among the 14 studies comparing the C versus the A allele (P=0.09) for A1298C polymorphism. Thus, the random effects model is more appropriate than the fixed model. The C allele tends to decrease the risk of CRC, but no significant association of the C allele with CRC susceptibility was found in a worldwide population (Fig. 2A, P=0.08, OR=0.93, 95% CI 0.85–1.01) and in an Asian population (P=0.2, OR=0.89, 95% CI 0.74–1.06, P=0.03 for heterogeneity). No heterogeneity (P=0.22) existed among the studies of Caucasian populations, and the fixed model did not reveal an association between 1298C and CRC risk (P=0.08, OR=0.90, 95% CI 0.79–1.01). Evidence for an association with CRC was discerned upon comparing the 1298CC genotype with the 1298AA genotype in a worldwide population (P=0.005, OR=0.80, 95% CI 0.69–0.94, P=0.18 for

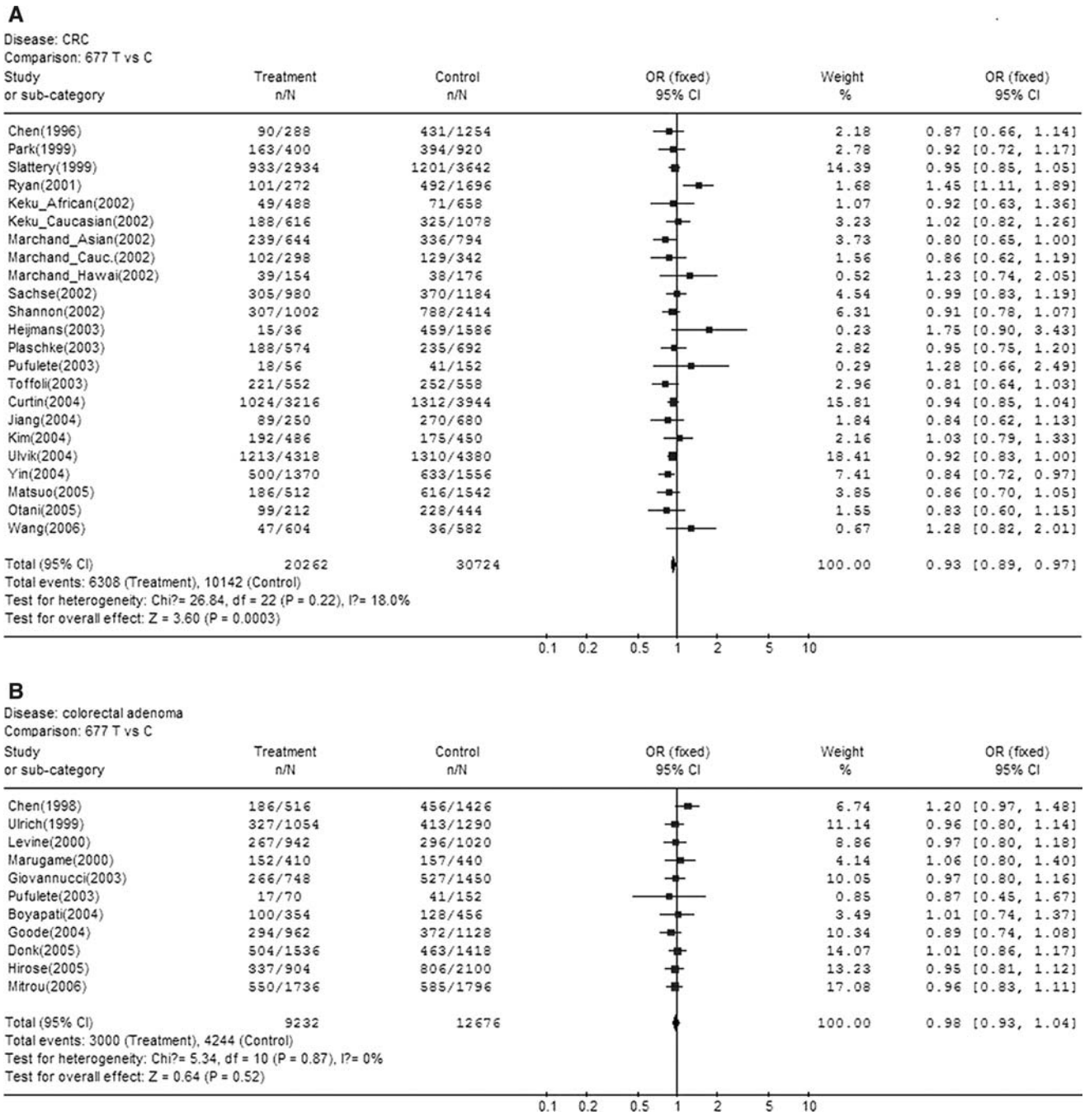

Fig. 1A–B Overall meta-analysis for C677T polymorphism (T vs. C allele) in CRC and colorectal adenoma. Point estimates of the OR for each study and the accompanying 95% CI values obtained with a fixed effects model are shown. **A** Analysis of the comparison with CRC, and **B** analysis of the comparison with colorectal adenoma. *n* indicates the total number of T or C alleles, *N* indicates the total number of T plus C alleles

heterogeneity) and a Caucasian subgroup (P=0.04, OR=0.74, 95% CI 0.55–0.98, P=0.23 for heterogeneity). No association with 1298CC that decreases CRC risk in Asians was found using a random-effects model (P=0.35, OR=0.78, 95% CI 0.46–1.33, P=0.05 for heterogeneity) existed in the homozygote contrast. Importantly, the 1298C allele also tends to decrease CRC risk in a recessive genetic model worldwide (P=0.005, OR=0.81, 95% CI 0.70–0.94, P=0.40 for heterogeneity). This effect is only significant in the Caucasian subgroup (P=0.04, OR=0.75, 95% CI 0.57–0.99, P=0.35 for heterogeneity), not in Asians (P=0.17, OR=0.81, 95% CI 0.60–1.09, P=0.11 for heterogeneity) using a fixed effects model.

Table 2 Summary of ORs for various genetic contrasts performed when investigating the association of C677T and A1298C polymorphisms with CRC and colorectal adenoma

Contrast	Comparisons (study numbers)	Random effects OR (95% CI)	Fixed effects OR (95%CI)	*P* value for heterogeneity	*P* value for significance with fixed effects model
C677T in CRC					
T vs. C	All (23)	0.93 (0.89–0.97)	0.93 (0.89–0.98)	0.22	0.004
	Caucasians (9)	0.93 (0.88–0.99)	0.93 (0.88–0.99)	0.52	0.03
	Asian (8)	0.87 (0.80–0.95)	0.87 (0.81–0.95)	0.61	0.001
TT vs. CC	All (23)	0.83 (0.74–0.93)	0.82 (0.75–0.89)	0.14	<0.00001
	Caucasians (9)	0.94 (0.75–1.19)	0.87 (0.75–1.01)	0.05	0.06
	Asian (8)	0.71 (0.60–0.85)	0.72 (0.60–0.85)	0.75	0.0002
TT vs. (CT + CC)	All (23)	0.86 (0.76–0.96)	0.83 (0.76–0.90)	0.06	<0.0001
	Caucasians (9)	1.02 (0.79–1.32)	0.91 (0.79–1.04)	0.006	0.16
	Asian (8)	0.75 (0.64–0.88)	0.75 (0.64–0.88)	0.71	0.0005
(TT + CT) vs. CC	All (23)	0.94 (0.89–1.00)	0.95 (0.90–1.00)	0.26	0.04
	Caucasians (9)	0.92 (0.84–1.00)	0.92 (0.85–1.00)	0.67	0.04
	Asian (8)	0.89 (0.79–1.00)	0.89 (0.79–1.00)	0.75	0.05
A1298C in CRC					
C vs. A	All (14)	0.93 (0.85–1.01)	0.94 (0.88–0.99)	0.09	0.03
	Caucasians (5)	0.91 (0.78–1.06)	0.90 (0.79–1.01)	0.22	0.08
	Asian (6)	0.89 (0.74–1.06)	0.91 (0.82–1.02)	0.03	0.1
CC vs. AA	All (14)	0.80 (0.65–0.98)	0.80 (0.69–0.94)	0.18	0.005
	Caucasians (5)	0.76 (0.53–1.09)	0.74 (0.55–0.98)	0.23	0.04
	Asian (6)	0.78 (0.46–1.33)	0.77 (0.57–1.05)	0.05	0.1
CC vs. (CA + AA)	All (14)	0.81 (0.69–0.96)	0.81 (0.70–0.94)	0.4	0.005
	Caucasians (5)	0.76 (0.57–1.03)	0.75 (0.57–0.99)	0.35	0.04
	Asian (6)	0.83 (0.52–1.31)	0.81 (0.60–1.09)	0.11	0.17
(CC + CA) vs. AA	All (14)	0.95 (0.87–1.03)	0.95 (0.88–1.03)	0.32	0.23
	Caucasians (5)	0.92 (0.78–1.08)	0.92 (0.78–1.08)	0.44	0.3
	Asian (6)	0.89 (0.74–1.06)	0.91 (0.81–1.04)	0.11	0.17
C677T in adenoma					
T vs. C	All (11)	0.98 (0.93–1.04)	0.98 (0.93–1.04)	0.87	0.52
	Caucasians (4)	0.96 (0.88–1.05)	0.96 (0.88–1.05)	0.79	0.35
	Asian (2)	0.98 (0.85–1.13)	0.98 (0.85–1.13)	0.52	0.78
TT vs. CC	All (11)	0.97 (0.86–1.11)	0.97 (0.85–1.11)	0.9	0.67
	Caucasians (4)	0.91 (0.74–1.12)	0.91 (0.74–1.12)	0.88	0.38
	Asian (2)	1.01 (0.75–1.35)	1.01 (0.75–1.35)	0.45	0.95
TT vs. (CT + CC)	All (11)	0.99 (0.88–1.12)	0.99 (0.88–1.12)	0.93	0.86
	Caucasians (4)	0.93 (0.76–1.13)	0.93 (0.76–1.13)	0.88	0.45
	Asian (2)	1.06 (0.81–1.39)	1.06 (0.81–1.39)	0.46	0.66
(TT + CT) vs. CC	All (11)	0.97 (0.90–1.05)	0.97 (0.90–1.05)	0.84	0.45
	Caucasians (4)	0.95 (0.85–1.07)	0.95 (0.85–1.07)	0.72	0.43
	Asian (2)	0.93 (0.77–1.13)	0.93 (0.77–1.13)	0.68	0.47
A1298C in adenoma					
C vs. A	All (3)	1.09 (0.94–1.27)	1.09 (0.97–1.22)	0.24	0.15
CC vs. AA	All (3)	1.22 (0.72–2.08)	1.18 (0.91–1.53)	0.09	0.21
CC vs. (CA + AA)	All (3)	1.18 (0.70–1.99)	1.14 (0.89–1.46)	0.08	0.3
(CC + CA) vs. AA	All (3)	1.10 (0.95–1.28)	1.10 (0.95–1.28)	0.68	0.2

Only three studies reported A1298C polymorphism in colorectal adenoma, one for an unknown population and the other two for populations of Caucasian descent. Ethnic subgroup analysis was not performed due to limited studies. No association was found between A1298C and colorectal adenoma in a worldwide population when comparing the A and C alleles (Fig. 2B, *P*=0.15, OR=1.09, 95% CI 0.97–1.22, *P*=0.24 for heterogeneity), as well as for other genetic contrasts (Table 2).

Sensitivity analysis

Sensitivity analysis was performed by sequential omission of individual studies from various contrasts performed on a worldwide population and on Cauca-

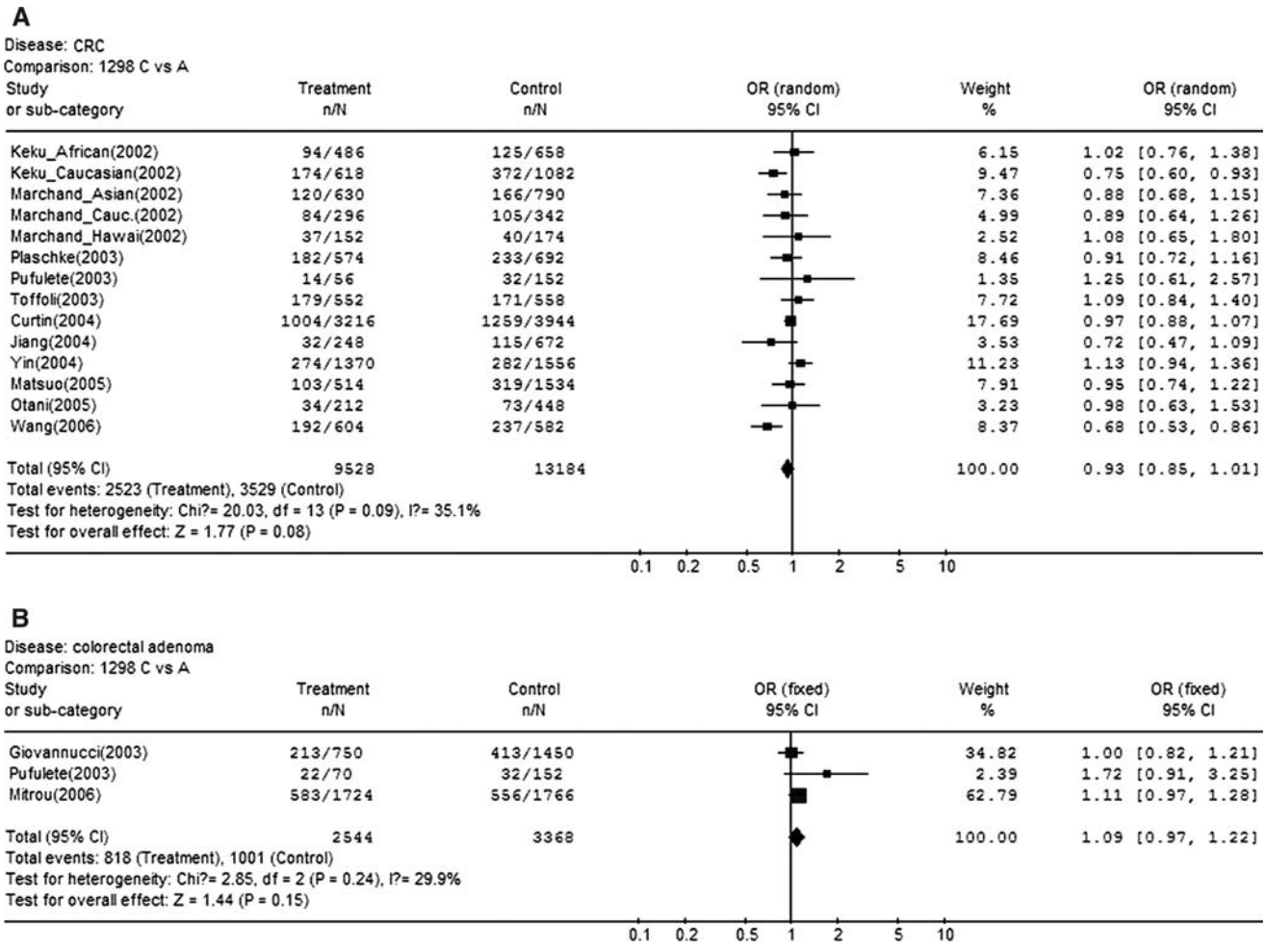

Fig. 2A–B Overall meta-analysis for A1298C polymorphism (C vs. A allele) in CRC and colorectal adenoma. Point estimates of the OR values for the studies are shown, as well as the accompanying 95% CI values obtained using a random effects model in (**A**; analysis of the comparison with CRC) and using a fixed effects model in (**B**; analysis of the comparison with colorectal adenoma). *n* indicates the total number of C or A alleles, *N* indicates the total number of C plus A alleles

sian or Asian subgroups. The pooled ORs (including 95% CI) were consistently below 1.0 in all of the comparisons for the three groups (worldwide population, Caucasian, Asian), with either fixed or random effect models applied, except for the dominant genetic model of C677T. The results did not alter the protecting role exhibited by the 677T allele against the CRC risk when using a recessive genetic model, indicating that the significance of the pooled ORs was not excessively influenced by any single study (data not shown). When individual studies were sequentially omitted for A1298C under homozygote and recessive contrasts in the worldwide population and for Caucasians, the pooled ORs were consistently below 1.0, suggesting that the protecting role exhibited by 1298C against the CRC risk when using a recessive genetic model is not affected by a single study. The associations of the C677T and A1298C polymorphisms with colorectal adenoma did not change during the sensitivity analysis.

Publication bias

A funnel plot for the comparison of the 677C allele with the 677T allele in the OR analysis and Egger's test provided no evidence for funnel plot symmetry for both colorectal adenoma (t=0.42, P=0.69) and CRC (t=1.50, P=0.15). Similarly, no publication bias was detected for the A versus C allele contrast of A1298C polymorphism for both colorectal adenoma (t=–0.36, P=0.72) and CRC (t=0.87, P=0.54).

Discussion

This meta-analysis examined the association of *MTHFR* C677T and A1298C polymorphisms with

susceptibility to CRC and colorectal adenoma. Little et al. (2003) found a protective effect of *MTHFR* C677T against CRC risk using a meta-analysis on 14 studies in 2003, including Ma et al. (1997), which deviated from HWE. A protective effect of *MTHFR* A1298C against colorectal cancer risk was also found using six studies. No association of *MTHFR* C677T with colorectal adenoma was found in their meta-analysis using six studies. Our meta-analysis results are consistent with those of Little et al. (2003), which were obtained by recruiting more up-to-date studies. We also investigated the association of *MTHFR* A1298C with colorectal adenoma risk. Importantly, we analyzed the effect of C677T and A1298C on CRC and colorectal adenoma risk by stratifying the ethnic populations studied.

A significant association between the *MTHFR* C677T polymorphism and CRC in a worldwide population for the overall effect of allele T versus C was found. Subgroup analysis suggested 677C polymorphism was a protecting factor against susceptibility to CRC for Asians when either homozygous (TT vs. CC) or recessive genetic model contrasts were performed. Significant associations were found between *MTHFR* A1298C polymorphism and CRC for the homozygote contrast and recessive genetic models. However, the Asian population does not show an association in the homozygote and recessive genetic analysis, in contrast to Caucasians, which retain the protecting effect of the 1298C against CRC risk. Thus, both the 677T allele and the 1298C allele had a decreased effect on CRC risk under the recessive genetic model, but the effect may be more pronounced in Asians and Caucasians, respectively. We investigated five studies which provided case and control numbers of the nine combined genotypes of C677T and A1298C (Curtin et al. 2004; Jiang et al. 2004; Keku et al. 2002; Plaschke et al. 2003; Yin et al. 2004). The probabilities of 677TC + 1298CC, 677TT + 1298AC, and 677TT + 1298CC genotypes were very rare from 3,243 cases and 4,198 controls of those studies. Ogino and Wilson (2003) calculated the haplotype frequencies of the C677T and A1298C polymorphisms in pooled general populations derived from data published in 16 articles. They found that most 677T and 1298C alleles were associated with the 1298A and 677C alleles, respectively. There may be an increased frequency of the very rare *cis* 677T/1298C haplotype in some parts of the UK and Canada, possibly due to a founder effect. Chen et al. (2002) reported the linkage disequilibrium between C677T and A1298C polymorphisms in *MTHFR*. Therefore, the effects on the CRC risk are independent for the two polymorphisms; both the 677T and 1298C alleles may be causal variants.

The 677T allele and the 1298C allele may decrease the risk of CRC, but they show no association with the risk of colorectal adenoma in a worldwide population and in the subgroups in our meta-analysis. The results suggest that the two polymorphisms may have a protecting effect, preventing colorectal adenoma from transforming into malignant CRC, since colorectal adenomas are precursor lesions of colorectal cancer (Fearon and Vogelstein 1990; Peipins and Sandler 1994). It is possible that *MTHFR* polymorphisms could modify the association between diet and colorectal adenomas. However, various results have been reported and no conclusions are available as yet. The *MTHFR* TT genotype in combination with a low folate status may be a risk factor for colorectal adenomas (Ulrich et al. 1999; Levine et al. 2000), or there may be no interaction (van den Donk et al. 2005).

Reasons for the conflicting results where *MTHFR* C677T and A1298C play different roles in different studies may be genetic heterogeneity in different populations and clinical heterogeneity in different studies. Potentially, differences in patient populations (e.g., in terms of age and years from onset, gender difference, and lifestyle) might cause different results. Shannon et al. (2002) showed that the *MTHFR* 677TT genotype conferred an increased risk of colorectal cancer on subjects older but not younger than 70 years. The risk effect may be dependent on gene methylation, which is affected by folate intake and its interaction with other risk factors. Chen et al. (1996) suggested a possible interaction between the *MTHFR* C677T polymorphism and folate status in CRC. TT genotype was protective in folate-replete subjects, whereas the combination of TT and low folate status conferred no protection, and even increased risk. Otani et al. (2005) reported no association between *MTHFR* C677T and A1298C–nutrient interaction in CRC, including vitamin B6, vitamin B12 and folate. Moreover, the association of alcohol consumption with colorectal cancer risk has been related to its anti-folate effects and subsequent effects on DNA methylation (Freudenheim et al. 1991; Giovannucci et al. 1993). Thus, diet, particularly low folic acid intake, can modify the effects of the MTHFR polymorphisms. However, no definite conclusion about the relation between folate intake and CRC risk has been possible so far. More careful stratification analysis that takes into account the clinical character and diet, smoking status and alcohol consumption are needed.

The 677T and 1298C SNPs in the *MTHFR* gene both decrease the activity of the enzyme, leading to hyperhomocysteinemia, particularly in folate-deficient states (Frosst et al. 1995; van der Put et al. 1998).

Elevated plasma homocysteine concentration had been suggested to be a risk factor for schizophrenia (Muntjewerff et al. 2006), coronary heart disease (Klerk et al. 2002), and stroke (Casas et al. 2005), particularly in low folate status. 677TT homozygotes show significantly greater mean homocysteine and risks of those three diseases than people who are 677CC homozygotes. However, Lewis et al. (2005) found no strong association of the *MTHFR* 677T polymorphism with coronary heart disease in Europe, North America, or Australia using a meta-analysis, which might due to the higher folate intake in North America and Europe. Here, folate provides one-carbon groups for the methylation of homocysteine to form methionine, which then decreases the concentration of homocysteine (Hankey and Eikelboom 2005). Zintzaras recently used meta-analysis to examine the association of *MTHFR* C677T and A1298C polymorphisms with breast cancer and gastric cancer, respectively (Zintzaras 2006a, 2006b). No association was found with breast cancer, but an increased risk from *MTHFR* 677T and 1298C was indicated for gastric cancer, in contrast to our meta-analysis results which indicate that *MTHFR* 677T and 1298C might be protective against CRC. Decreased levels of methyltetrahydrofolate may adversely affect the methylation of oncogenes and tumor suppressor genes, contributing to carcinogenesis. Also, depletion of methylenetetrahydrofolate interferes with thymidylate biosynthesis, which may lead to an accumulation of deoxyuridylate DNA, and subsequent removal of this abnormal base may destroy the integrity of DNA (Houlston and Tomlinson 2001). Thus, the balance between DNA synthesis and DNA methylation, which is determined by the *MTHFR* polymorphisms, may influence the cancer risk, which is also affected by folate intake.

Only two of the studies involved in our meta-analysis investigated gene–gene interactions. Ulvik et al. (2004) found no association of *MTHFR* C677T and *MTR* (methionine synthase) A2756G with CRC risk, and Hirose et al. (2005) revealed a decreased risk of CRC from *ALDH2*2* and *MTHFR* 677T. More research on gene–gene interactions such as those involving *MTR*, thymidylate synthase, and *ALDH2* will provide a more comprehensive insight into the associations studied here.

In conclusion, our meta-analysis (along with other studies) suggests that the *MTHFR* 677T and 1298C alleles have no association with colorectal adenoma risk, and they may both provide protecting effects against CRC risk in a recessive genetic model; however, the protecting effect of 677T was only significant in Asians, while 1298C was only significant in Caucasians. Whether other *MTHFR* polymorphisms provide protective effects against CRC risk in different populations in a recessive genetic model requires further investigation. More studies or large case-control studies should be performed to clarify the possible roles of C677T and A1298C in CRC.

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