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Review Article

Folate Intake, *MTHFR* Polymorphisms, and the Risk of Colorectal Cancer: A Systematic Review and Meta-Analysis

Deborah A. Kennedy,[1,2] Seth J. Stern,[1,3] Ilan Matok,[1] Myla E. Moretti,[1] Moumita Sarkar,[1] Thomasin Adams-Webber,[1] and Gideon Koren[1,2,3]

[1] *The Motherisk Program, Division of Clinical Pharmacology and Toxicology, The Hospital for Sick Children, 555 University Avenue, Toronto, ON, Canada M5G 1X8*
[2] *Leslie Dan Faculty of Pharmacy, University of Toronto, 144 College Street, Toronto, ON, Canada M5S 3M2*
[3] *Department of Pharmacology and Toxicology, Faculty of Medicine, University of Toronto, Medical Sciences Building, 1 King's College Circle, Toronto, ON, Canada M5S 1A8*

Correspondence should be addressed to Gideon Koren, gidiup_2000@yahoo.com

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Background. The objective was to determine whether relationships exist between the methylene-tetrahydrofolate reductase (*MTHFR*) polymorphisms and risk of colorectal cancer (CRC) and examine whether the risk is modified by level of folate intake. *Methods*. MEDLINE, Embase, and SCOPUS were searched to May 2012 using the terms "folic acid," "folate," "colorectal cancer," "methylenetetrahydrofolate reductase," "*MTHFR*." Observational studies were included which (1) assessed the risk of CRC for each polymorphism and/or (2) had defined levels of folate intake for each polymorphism and assessed the risk of CRC. *Results*. From 910 references, 67 studies met our criteria; hand searching yielded 10 studies. The summary risk estimate comparing the *677*CT versus CC genotype was 1.02 (95% CI 0.95–1.10) and for *677*TT versus CC was 0.88 (95% CI 0.80–0.96) both with heterogeneity. The summary risk estimates for A1298C polymorphisms suggested no reduced risk. The summary risk estimate for high versus low total folate for the *677*CC genotype was 0.70 (95% CI 0.56–0.89) and the *677*TT genotype 0.63 (95% CI 0.41–0.97). *Conclusion*. These results suggest that the *677*TT genotype is associated with a reduced risk of developing CRC, under conditions of high total folate intake, and this associated risk remains reduced for both *MTHFR 677* CC and TT genotypes.

1. Introduction

Worldwide, colorectal cancer (CRC) is the third most frequently diagnosed cancer in males and the second in females [1]. Australia and New Zealand, Europe and North America have the highest incidence rates of CRC worldwide, and Africa and South-Central Asia, the lowest [1, 2]. Over 75% of CRCs occur sporadically, with only 25% of patients having a family history of CRC [3].

Folate insufficiency has been suggested as one of the possible mechanism for CRC development and progression. DNA strand breaks, impaired DNA methylation and repair have been associated with folate deficiency and CRC [4–7]. There are many enzymes involved with folates and one-carbon metabolism; however, the *methylene tetrahydrofolate reductase (MTHFR)* enzyme is a key enzyme responsible for determining whether reduced folates are directed towards DNA methylation pathways or pyrimidine or purine synthesis [8]. In 1995, a variant of *MTHFR* enzyme was identified which causes a substitution of C to T at nucleotide 677 [9]. The *MTHFR C677T* homozygous variant (TT genotype) is thermolabile, and its activity is reduced by 70% compared to the wild type (CC genotype) [10]. This reduced enzyme activity causes an accumulation of plasma homocysteine and higher rates of thymidylate synthesis [10, 11].

The distribution of the TT genotype varies from country to country. In Europe, there would appear to be a north-south gradient with the distribution of the TT genotype lowest in the north [12, 13] while in Asia, the frequency is highest in China and lowest in India [12, 14–18]. In North

America, African Americans have a much lower TT genotype frequency versus Caucasians [19]. Individuals with this variant are thought to be at greater risk for a number of diseases including cardiovascular disease, acute lymphocytic leukemia, and neural tube defects [10]. Some published studies have suggested that those with the TT genotype have a reduced risk of CRC versus those with the CC genotype (wild type) [20–28]; however, other studies have found an increased risk [29–31].

A second variant of the *MTHFR* enzyme, with a substitution of A to C at nucleotide 1298, has also been identified. Unlike the *MTHFR C677T* polymorphism, the enzyme activities of the variants of *MTHFR A1298C* polymorphism are not thermolabile, but the enzyme activity is reduced by approximately 40% of the wild type (AA genotype) in the variant genotype. Altered homocysteine levels have not been found in individuals with these variants [32]. The prevalence of the *1298*CC genotype varies, with the homozygous genotype found in 7–12% of Caucasians, in Europeans, 4–12%, while in China, Japan, and Hawaiian studies the prevalence ranged between 1 and 4% [32, 33].

The objective of this effort was to conduct a systematic review and meta-analysis of the published data to determine whether relationships exist between the various *MTHFR* polymorphisms and the incidence of CRC. A secondary objective was to examine whether there exists a relationship between the level of folate intake for each *MTHFR* genotype and the risk of CRC.

2. Methods and Materials

2.1. Inclusion Criteria. We selected observational studies reporting on the polymorphisms of the *MTHFR C677T* and/or *A1298C* genes and the associated risk of CRC, colon, or rectal cancer in adult populations. Studies were also included if they reported on folate exposure (dietary or total) with at least two levels of folate intake and the associated rates of colorectal, colon and/or rectal cancer by genotype. Studies were excluded if they did not provide the information necessary to determine an odds ratio and 95% confidence interval for each genotype. No restrictions were placed on language of publication or country of study.

2.2. Search Strategy. The databases MEDLINE, Embase, and Scopus on the OVID platform were searched from inception to May 2012. Both database-specific subject headings and text words were searched using the terms "folic acid" OR "folate" and "colorectal cancer" and "colorectal neoplasms" AND "methylenetetrahydrofolate reductase or *MTHFR* or C667T" limiting the results to humans only. The results of the search in each of the three databases were placed in a bibliography tool, and, in order to ensure blinding, an extract of author, title, and year information was made and uploaded into a spreadsheet for the purposes of title review. Title review was conducted by one reviewer (DAK) blinded to the journal of publication, place of research, and results, to determine which study articles to retrieve. The methods section of the selected journal articles were retrieved by other team members (MS and IM) not responsible for reviewing the journal articles. The method sections were reviewed by two independent reviewers (DAK, SJS) blinded to the journal of publication, place of research, and results as to their meeting the inclusion criteria. In case of disagreement between the two reviewers, a third reviewer served as a tiebreaker (GK). Previous reviews were also hand searched to identify other relevant publications to include.

2.3. Data Extraction. Data extraction was carried out by one reviewer and independently checked for accuracy by a second reviewer. Data collected included the type of study, location, study inclusion and exclusion criteria, case and comparator group size, folate intake levels, odds ratio or risk ratio, the number, for both case and control, and percentage frequency of each genotype, relevant adjustments, and conclusions. The genotype distribution of the control group was evaluated for agreement with the Hardy-Weinberg equilibrium (HWE) using chi-squared with a significant level of 0.05 and the results incorporated into Table 1.

The Downs and Black scoring instrument was used to determine the quality of the studies included in this paper. The Down and Black scoring tool provides a means to assess the quality of a study based on 5 subscales (1) reporting of the study results, (2) external validity for the purposes of assessing generalizability of the findings, (3) bias in measurement and outcomes, (4) bias in the selection of study subjects, and (5) the power of the study [79]. The score was independently calculated for each study by two team members. Disagreements were resolved by consensus. The last question on the Downs and Black tool relates to the power of the study. If *a priori* power calculation was reported in the paper, this question was scored with a one, otherwise, zero was scored.

2.4. Statistical Analysis. The meta-analysis for the genotype risk comparisons was performed using the inverse variance method under a random effects model, odds ratios (ORs) along with 95% confidence intervals (CIs) were used for the case control studies according to the DerSimonian and Laird method [80]. All identified studies with available data were included in the summary effect estimate for each genotype combination. For the meta-analysis of the risk of CRC associated with genotype, the wild type (*677*CC *or 1298*AA) was used as the reference group, and comparisons were made to either the heterozygous (*677*CT *or 1298*AC) or homozygous variant type (*677*TT *or 1298*CC). If studies grouped genotypes together for comparison purposes, or did not report ORs and 95% confidence intervals and the raw numbers were available in the paper, unadjusted ORs and associated 95% confidence intervals were calculated according to the method described by Silman and MacFarlane [81]. These are identified in Table 1 as "OR calculated, no adjustments" in the column titled Adjustments. The meta-analyses were performed using Review Manager 5.1 Software [82].

The meta-analyses for the comparison of high versus low folate intake and the associated risk of CRC were performed using the inverse variance method under a random effects

Table 1

(a) Characteristic of studies included in the systematic review and meta analysis.

Study	Country	Year	Study design	Source of controls	Recruitment period	Cancer	Sex	Case/control	Distribution of *MTHFR C677T* genotype in controls CC (%)	CT (%)	TT (%)	HWE (yes/no)	Distribution of *MTHFR A1298C* genotype in controls AA (%)	AC (%)	CC (%)	HWE (yes/no)	Adjustments
Park et al. [34]	South Korea	1999	Case control	Healthy persons	Not reported	CRC	Both	200/460	30.4	53.5	16.1	No					Calculated OR, no adjustments
Delgado-Enciso et al. [15]	Mexico	2001	Case control	Not reported	1997	CRC	Both	74/110	30.9	47.3	21.8	Yes					Calculated OR, no adjustments
Keku et al. [35]	USA	2002	Case control	Healthy persons	1996–2000	Colon-Cauc	Both	555/875	49.2	41.4	9.4	Yes	52.5	38.5	9.3	Yes	Adjusted for sampling fraction, age, gender, and energy intake
Le Marchand et al. [23]	USA	2002	Matched case control	Healthy persons	1994–1998	CRC	Both	727/727	39.2	45	15.8	Yes	58.2	36.0	5.8	No	Adjusted for age, gender, ethnicity, pack years of cigarette smoking, lifetime recreational physical activity, lifetime aspirin use, BMI 5 years ago, years of schooling, and intakes of nonstarch polysaccharides from vegetables and calcium from foods and supplements
Matsuo et al. [16]	Japan	2002	Case control	Hospital patients	1999	CRC colon rectal	Both	142/241 72/241 70/241	33.6	51.5	14.9	Yes	65.1	31.1	3.7	Yes	Adjusted for age
Shannon et al. [36]	Australia	2002	Case control	Healthy persons	1985–1998	CRC	Both	501/ 1,207	44.2	46.4	9.4	Yes					Calculated OR, no adjustments
Plaschke et al. [37]	Germany	2003	Case control	Healthy persons	Not reported	CRC	Both	287/346	43.1	45.9	11	Yes	44.5	43.6	11.8	Yes	Adjusted for gender
Pufulete et al. [38]	United Kingdom	2003	Case control	Hospital patients	2000-2001	CRC	Both	304/352	54	38	8	Yes	61.8	34.2	3.9	Yes	Adjusted for age, gender, BMI, smoking, and ROH intake.
Toffoli et al. [39]	Italy	2003	Case control	Healthy persons	1999-2000	Colon	Both	276/279	29.7	50.2	20.1	Yes	47.7	43.4	9	Yes	Calculated OR, no adjustments
Jiang et al. [40]	China	2004	Case control	Healthy person	1990–2002	CRC	Both	126/343	39.1	42.4	18.5	No	67.6	30.7	1.8	Yes	Adjusted for age and sex

(a) Continued.

Study	Country	Year	Study design	Source of controls	Recruitment period	Cancer	Sex	Case/control	Distribution of *MTHFR C677T* genotype in controls CC (%)	CT (%)	TT (%)	HWE (yes/no)	Distribution of *MTHFR A1298C* genotype in controls AA (%)	AC (%)	CC (%)	HWE (yes/no)	Adjustments
Miao et al. [41]	China	2005	Case control	Healthy persons	1999–2002	CRC	Both	198/420	31.7	47.9	20.5	Yes	67.1	31.4	1.4	No	None reported
Ulvik et al. [20]	Norway	2004	Nested case control	Healthy persons	1992-1991	CRC	Both	2,168/ 2,168	49.9	40.4	9.7	Yes					Adjusted for age and gender
Yin et al. [21]	Japan	2004	Case control	Hospital patients	2000–2003	CRC	Both	685/778	35.7	47.2	17.1	Yes	66.2	31.4	2.4	Yes	Adjusted for gender. 5-year age class, area and alcohol use
Jiang et al. [42]	China	2005	Nested case control	Healthy persons	1989-1990	Colon rectal	Both	52/338 72/338	39.5	42.2	18.3	No	67.5	30.7	1.8	No	Adjusted for gender, age, folate, methionine, total energy intake, smoking status, and drinking status
Landi et al. [43]	Spain	2005	Case control	Hospital patients	1996–1998	CRC	Both	359/320	35.3	45	19.7	Yes	53.3	39.8	6.9	Yes	Adjusted for age and sex
Matsuo et al. [44]	Japan	2005	Matched case control	Hospital patients	2001–2004	CRC	Both	257/771	37.5	45.1	17.3	Yes	62.5	33.5	4.0	Yes	Adjusted for age, sex, referral patterns, smoking BMI, physical, exercise and family history of CRC
Otani et al. [45]	Japan	2005	Matched case control	Hospital patients	1998–2002	CRC	Both	107/224	23	51.4	25.6	Yes	69.6	28.1	2.2	Yes	Matching factors and adjusted for smoking, alcohol consumption, BMI, and total dietary fiber intake
Le Marchand et al. [46]	USA (Hawaii and California)	2005	Nested case control	Healthy persons	1993–1996	CRC colon rectal	Both	822/ 2,021	48.9	38.5	12.6	No					Adjusted for age, gender, and ethnicity
Battistelli et al. [47]	Italy	2006	Case control	Healthy controls	Not reported	CRC	Both	93/100	30	51	29	Yes					Calculated OR, no adjustments
Van Guelpen et al. [24]	Sweden	2006	Nested case control	Healthy persons	1985–2002	CRC	Both	226/437	52.8	38.7	8.5	Yes	45.9	42.0	12.1	Yes	Adjusted for BMI, smoking, recreational and occupational physical activity, and alcohol intake

(a) Continued.

Study	Country	Year	Study design	Source of controls	Recruitment period	Cancer	Sex	Case/control	Distribution of *MTHFR C677T* genotype in controls CC (%)	CT (%)	TT (%)	HWE (yes/no)	Distribution of *MTHFR A1298C* genotype in controls AA (%)	AC (%)	CC (%)	HWE (yes/no)	Adjustments
Wang et al. [48]	India	2006	Case control	Healthy persons	1999–2001	CRC colon rectal	Both	435/340	87.6	12.4	0	Yes	36.1	46.4	17.5	Yes	Adjusted for gender, age household income, education, religion, mother tongue, smoking, drinking, chewing, and vegetarianism
Lima et al. [49]	Brazil	2007	Case control	Healthy persons	1999–2001	CRC	Both	102/300	47.7	42.3	10	Yes	63.7	31.0	5.3	Yes	Adjusted for age, gender, and race
Chang et al. [50]	Taiwan	2007	Matched case control	Hospital patients	2000-2001	CRC	Both	195/195	47.2	44.6	8.2	Yes	61.5	33.3	5.1	Yes	Matched on age and gender.
Curtin et al. [51]	USA	2007	Matched case control	Healthy persons	1991–1994	Colon	Both	916/ 1,972	45.0	43.5	11.5	Yes	47.1	41.9	11	Yes	Calculated OR, no adjustments
Hubner et al. [52]	United Kingdom	2007	Case control	Healthy controls	Not reported	CRC	Both	1,685/ 2,695	43.6	44.3	12.1	Yes					Adjusted for age, sex, family history, cancer location, stage, and grade
Jin et al. [53]	China	2007	Case control	Healthy controls	2002–2005	CRC	Both	449/672	31.4	48.4	20.2	Yes					Adjusted for age, sex, drinking, BMI, smoking, and family history
Murtaugh et al. [54]	USA	2007	Matched case control	Healthy persons	1997–2001	Rectal-Men Rectal-Women	Both Men Women	751/979	48.2 47.5	40.1 41.4	11.7 11.1	No	44.9	43.7	11.3	Yes	Adjusted for age, BMI, activity, energy, fiber, calcium, ibuprofen use, smoking, and other *MTHFR* genotype
Osian et al. [55]	Romania	2007	Matched case control	Hospital patients	2003–2005	CRC	Both	69/67	70.2	25.4	4.5	Yes	61.1	37.3	1.5	Yes	Matched on age and sex
Zeybek et al. [17]	Turkey	2007	Case control	Hospital patients	2003–2005	CRC	Both	52/144	44.4	45.1	10.4	Yes					Calculated OR, no adjustments
Cao et al. [56]	China	2008	Matched case control	Healthy persons	2000–2002	CRC	Both	315/371	32.7	49.5	17.8	Yes	64.4	32.1	3.5	Yes	Matched on ethnicity, sex, and age

(a) Continued.

Study	Country	Year	Study design	Source of controls	Recruitment period	Cancer	Sex	Case/control	Distribution of *MTHFR C677T* genotype in controls			HWE (yes/no)	Distribution of *MTHFR A1298C* genotype in controls			HWE (yes/no)	Adjustments
									CC (%)	CT (%)	TT (%)		AA (%)	AC (%)	CC (%)		
Küry et al. [57]	France	2008	Matched case control	Healthy persons	2002–2006	CRC	Both	1,023/1,121	40.8	45.9	13.3	Yes	51.5	39.5	9	Yes	Matched on age and sex
Lightfoot et al. [58]	United Kingdom	2008	Matched case control	Hospital patients	1997–2000	CRC	Both	468/734	45.8	46	8.3	Yes	48.6	43.7	7.8	Yes	Adjusted for gender and age
Mokarram et al. [59]	Iran	2008	Case control	Not reported	2003–2005	Colon	Both	151/81	49.4	38.3	12.3	Yes					Calculated OR, no adjustments
Sharp et al. [60]	United Kingdom (Scotland)	2008	Matched case control	Healthy persons	1998–2000	CRC	Both	264/408	43.2	44.9	11.9	Yes	44.9	39.8	15.2	No	Adjusted for age, gender, family history of CRC, physical activity, NAAID use, total energy intake, and type of dietary supplements
Theodoratou et al. [61]	Scotland	2008	Case control	Healthy persons	1999–2006	CRC	Both	2,028/2,722	45.3	45.0	11.5	Yes	45.8	44.1	10.1	Yes	Adjusted for age, sex, deprivation score, and family history risk
Zhang et al. [62]	China	2008	Matched case control	Hospital patients	2003–2005	CRC	Both	300/300	30.4	46.5	23.1	Yes	65.3	29.7	5	Yes	Adjusted for age, sex, education, family history, smoking, and drinking.
El Awady et al. [63]	Egypt	2009	Case control	Healthy persons	2004–2007	CRC	Both	35/68	65	29	6	Yes	38	54	8	Yes	None reported
Gallegos-Arreola et al. [30]	Mexico	2009	Case control	Healthy persons	2006–2008	CRC	Both	369/170	33.6	34.1	32.2	No					Calculated OR, no adjustments
Haghighi et al. [22]	Iran	2009	Case control	Hospital patients	2004–2007	CRC	Both	234/257	36.6	31.1	32.3	Yes					None reported
Iacopetta et al. [18]	Australia	2009	Matched case control	Healthy persons	2005–2007	Proximal distal CRC	Both	850/958	45	45	10	Yes					Matched on gender, age, socioeconomic status, country of birth, educational level, and smoking status

(a) Continued.

Study	Country	Year	Study design	Source of controls	Recruitment period	Cancer	Sex	Case/control	Distribution of *MTHFR C677T* genotype in controls CC (%)	CT (%)	TT (%)	HWE (yes/no)	Distribution of *MTHFR A1298C* genotype in controls AA (%)	AC (%)	CC (%)	HWE (yes/no)	Adjustments
Chandy et al. [14]	India	2010	Matched case control	Healthy persons	2006–2008	CRC	Both	100/86	76.7	22.1	1.2	Yes	25.6	58.1	16.3	Yes	Matched on age and gender
Cui et al. [28]	South Korea	2010	Case control	Hospital patients	2004–2008	CRC	Both	1,829/1,700	31.8	50.7	17.5	Yes					Adjusted for age and sex
Eussen et al. [64]	EPIC	2010	Nested case control	Healthy persons	1992–1998	CRC	Both	1,367/2,325	43.1	45.5	11.5	Yes	46.5	42.6	11.0	Yes	Calculated OR, no adjustments
Fernández-Peralta et al. [65]	Spain	2010	Matched case control	Healthy persons	1992–1996	CRC	Both	143/103	42.7	48.6	8.7	Yes	55.3	42.7	1.9	No	Matched on age and sex
Karpinski et al. [66]	Poland	2010	Case control	Healthy persons	Not reported	CRC	Both	186/140	51	39	10	Yes					Adjusted for age and sex
Komlósi et al. [67]	Hungary	2010	Case control	Healthy persons	2001–2007	Colon rectal	Both Both	476/461 479/478	47 47	40 41	13 12	Yes Yes					Adjusted for sex, age, and BMI Adjusted for sex, age, and BMI
Naghib alhossaini et al. [68]	Iran	2010	Case control	Not reported	Not reported	CRC	Both	151/230	42.4	53	4.6	Yes	42.5	45.7	11.8	Yes	Adjusted for age, sex, and smoking status
Promthet et al. [69]	Thailand	2010	Matched case control	Healthy persons	2002–2006	Colon	Both	130/130	72.3	23.8	3.9	Yes	41.5	54.6	3.9	No	Matched on age and sex
Wettergren et al. [70]	Sweden	2010	Case control	Healthy persons	1994–2004	CRC	Both	181/300	55.9	35.8	8.4	Yes					Calculated OR, no adjustments
Abuli et al. [71]	Spain	2011	Matched case control	Healthy person	2000-2001	CRC	Both	515/515	38.1	48	13.9	Yes					Matched on age and sex
Guimarães et al. [72]	Brazil	2011	Case control	Healthy persons	1992–2003	CRC	Both	113/188	48.9	42.0	9	Yes	67.6	26.1	6.4	No	Adjusted for age, sex, and ethnic origin
Jokić et al. [73]	Croatia	2011	Case control	Healthy persons	Not reported	Colon	Both	300/300	47.3	43.3	9.3	Yes	46.7	42.7	10.6	Yes	
Pardini et al. [25]	Czech Republic	2011	Case control	Hospital patients	2004–2006	CRC	Both	666/1,377	44.5	45.6	9.9	Yes	42.3	46.3	11.3	Yes	Adjusted for age and gender

(a) Continued.

Study	Country	Year	Study design	Source of controls	Recruitment period	Cancer	Sex	Case/control	Distribution of *MTHFR C677T* genotype in controls CC (%)	CT (%)	TT (%)	HWE (yes/no)	Distribution of *MTHFR A1298C* genotype in controls AA (%)	AC (%)	CC (%)	HWE (yes/no)	Adjustments
Kim et al. [26]	South Korea	2011	Case control	Hospital patients	Not reported	CRC	Both	67/53	28.3	39.6	32.1	Yes	67.9	30.2	1.9	Yes	None reported
Sameer et al. [74]	Kashmiri (India)	2011	Matched case control	Healthy persons	2008-2009	CRC	Both	86/160	75.6	16.9	7.5	No					None reported
Prasad and Wilkhoo [75]	India	2011	Case control	Healthy person	Not reported	CRC	Both	110/241	94.6	5.0	0.4	Yes					None reported
Zhu et al. [29]	China	2011	Case control	Healthy persons	2006–2008	CRC	Both	86/100	49.0	41.0	10	Yes					None reported
Kim et al. [27]	South Korea	2012	Case control	Hospital patients	1998–2004	CRC	Both	787/656	31.3	44.1	24.7	No					Adjusted for age, sex, family history, multivitamin use, BMI, smoking status, and total energy
Lee et al. [76]	USA	2012	Nested case control	Healthy persons	Health pro-fessionals follow-up study	CRC	Men	173/345	44	39.9	16	Yes	47.7	42.6	10.3	Yes	RR's reported, so OR's are calculated, no adjustments
Lee et al. [76]	USA	2012	Nested Case Control	Healthy persons	Physicians' health study	CRC	Men	240/408	47.7	37.2	15	Yes	45.8	42.2	12.1	Yes	RR's reported, so OR's are calculate, no adjustments
Lee et al. [76]	USA	2012	Nested Case Control	Healthy persons	Nurse Health Study	CRC	Women	189/377	46.7	39.7	13.6	Yes	51	38.3	10.7	Yes	RR's reported, so OR's are calculated no adjustments

AA: African American, BMI: body mass index, Cauc: Caucasian CRC: colorectal cancer, HWE: Hardy Weinberg equilibrium, NSAID: nonsteroidal anti-inflammatory drug, OCP: oral contraceptive pill.

(b) Summary of cohorts studies.

Study	Country	Year	Study design	Source of control	Recruitment period	Cancer	Sex	Case/control	Follow-up period	Incidence rate ratio (RR) of CRC (95% CI) CT versus CC	TT versus CC	AC versus AA	CC versus AA	
De Vogel et al. [77]	Netherlands	2009	Cohort	Healthy persons	Recruited in 1986	CRC	Both	689/1,793	7.3 years	1.23 (1.02–1.50)	0.80 (0.56–1.15)	0.89 (0.72–1.09)	1.05 (0.79–1.38)	Adjusted for age and sex
Heijmans et al. [78]	Netherlands	2003	Cohort	Elderly healthy men	Recruited in 1985	CRC	Both	18/793	10 years	1.16 (0.41–3.30)	3.65 (1.97–12.5)			Adjusted for age

model, odds ratios (ORs) along with 95% confidence intervals (CIs) were calculated according to the DerSimonian and Laird method [80]. All identified studies with available data were included in the summary effect estimate for each high versus low folate intake within a genotype. For those studies that compared folate intake by "quantile" and assessed the risk of CRC by genotype, many used the *677*CC or *677*CC/CT lowest folate intake quantile as the reference group to determine the OR for all genotypes and folate intake levels. For the purposes of this analysis, however, the desire was to compare the risk of CRC between the highest folate intake to lowest folate intake within a genotype. The method described by Hamling et al. and the associated MS Excel spreadsheet, which recalculates the adjusted odds ratios permitting alternative comparisons, were used to derive the ORs of highest compared to the lowest folate intake within the genotype [83, 84]. This analysis was performed using Microsoft Excel (Microsoft Corporation (2007), Redmond, WA, USA). An analysis of folate intake and CRC risk for the *MTHFR A1298C* gene was not possible due to an insufficient number of studies reporting on this data. In performing this analysis, the result from the highest "quantile" identified in the study was used to compare the lowest "quantile" in the study. Dietary folate intake for the lowest "quantile" ranged from a low of less than 115.6 to 406 mcg/day; the range for the highest was from 320 to 485 mcg/day or more. Although these ranges do overlap, they represent the highest and the lowest folate intake for the study population upon which the specific study odds ratios were derived. The meta-analyses were performed using Review Manager 5.1 Software [82].

Publication bias was assessed via the Begg and Mazumdar's rank correlation test, Egger's linear regression, and the Trim and Fill methods [85–87]. The assessment of publication bias was performed using the Comprehensive Meta-analysis (CMA) software (Biostat, Version 2.2, Englewood, NJ, USA) [88]. Summary effect estimates from CMA were compared with the RevMan results to ensure that they were both in agreement prior to executing the tests for publication bias.

Assessment of heterogeneity was performed using both Cochran's χ^2 and I^2. The Cochran's χ^2 test assesses whether the differences in results are due to chance only [89]. Heterogeneity exists when the P value is low, that is, $P < 0.10$ [89]. The I^2 statistic is the percentage of variability in the effect estimates that is due to heterogeneity rather than chance. An I^2 statistic value over 50% indicates that substantial heterogeneity may be present [89]. The analysis was performed using Review Manager 5.1 software [82].

Kruskal-Wallis was performed on the quality of the studies to determine whether there were differences in the quality of the studies based on the directionality of the outcome. For the purposes of this analysis, directionality was assessed as positive (statistical significant OR > 1), neutral (nonsignificant OR), or negative (statistical significant OR ≤ 1). IBM's SPSS for Windows version 17 was used for the analysis (IBM SPSS, Version 17, Chicago, IL, USA).

The Forest plots of the *MTHFR C677T* and *A1298C* (Figures 2 through 5) were sorted according to the percentage of the comparator genotype (either *677*CT, *677*TT, *1298*AC, *or 1298*CC) in the control group, from highest to lowest, while the remaining Forest plots (Figure 6) were organized by increasing year of publication.

3. Results

The pooled search resulted in 910 records. Of these 67 met our inclusion criteria, 10 studies were found on hand searching (Figure 1). Four identified studies were not included in the paper. In two studies, newborns comprised either all or part of the control group, which suggested that these studies were related to the determination of the prevalence of genotypes rather than risk of CRC since few newborns have had the opportunity to develop colorectal cancer [8, 92]. The remaining two studies did not report the separate case control numbers for each genotype; therefore, ORs could not be calculated for all genotypes; however the folate intake results, reported on in one of these studies, are included in the high versus low folate intake analysis [31, 93]. The majority of the studies included in the systematic review and meta-analysis were case control or nested case control studies, two cohort studies were identified (Table 1). The meta-analysis results presented here update two previously published meta-analyses on *MTHFR* polymorphisms and the risk of colorectal cancer, that of Taioli et al. 2009 meta-analysis on the *MTHFR C677T* polymorphisms and Kono and Chen's 2005 meta-analysis on the *MTHFR A1298C* polymorphisms [94, 95]. All case control studies, with available data, were included in the meta-analysis, regardless of the quality score.

Study results were reported from twenty-five countries: Asia (China, India, Japan, South Korea, Taiwan, and Thailand), Australia, Europe (EPIC Cohort (10 European Centers), Czech Republic, Croatia, France, Germany, Hungary, Italy, Norway, Poland, Romania, Spain, Sweden, and United Kingdom), Latin America (Mexico), Middle East (Egypt, Iran, and Turkey), South America (Brazil), and USA. Six papers were written in another language with an English abstract: five in Chinese: the other in Spanish [31, 40, 41, 53, 62, 93]. When duplicate studies were found, for example, Nurses' Health study and Health Professionals study, only the most recently published results were used in this analysis. There were five studies whose recruitment period was during the early days of folate fortification in USA; otherwise none of the studies were conducted in an environment of food fortification [35, 54, 76]. A blood sample was the most often used medium to assess genotype. There were two studies that used buccal samples as the tissue source for genotyping [18, 60].

3.1. Colorectal Cancer Risk and MTHFR C677T Genotype. For the comparison of *677*CT versus *677*CC, the summary risk estimate of the adjusted ORs was 1.02 (95% CI 0.95–1.10), $\chi^2 = 210.34$, df = 63, $P < 0.00001$, $I^2 = 70\%$ with significant heterogeneity (Figure 2). For the comparison of *677*TT versus *677*CC genotype, the summary risk estimate was 0.88 (95% CI 0.80–0.96) $\chi^2 = 132.66$, df = 61, $P = 0.00001$, $I^2 = 54\%$ with significant heterogeneity (Figure 3).

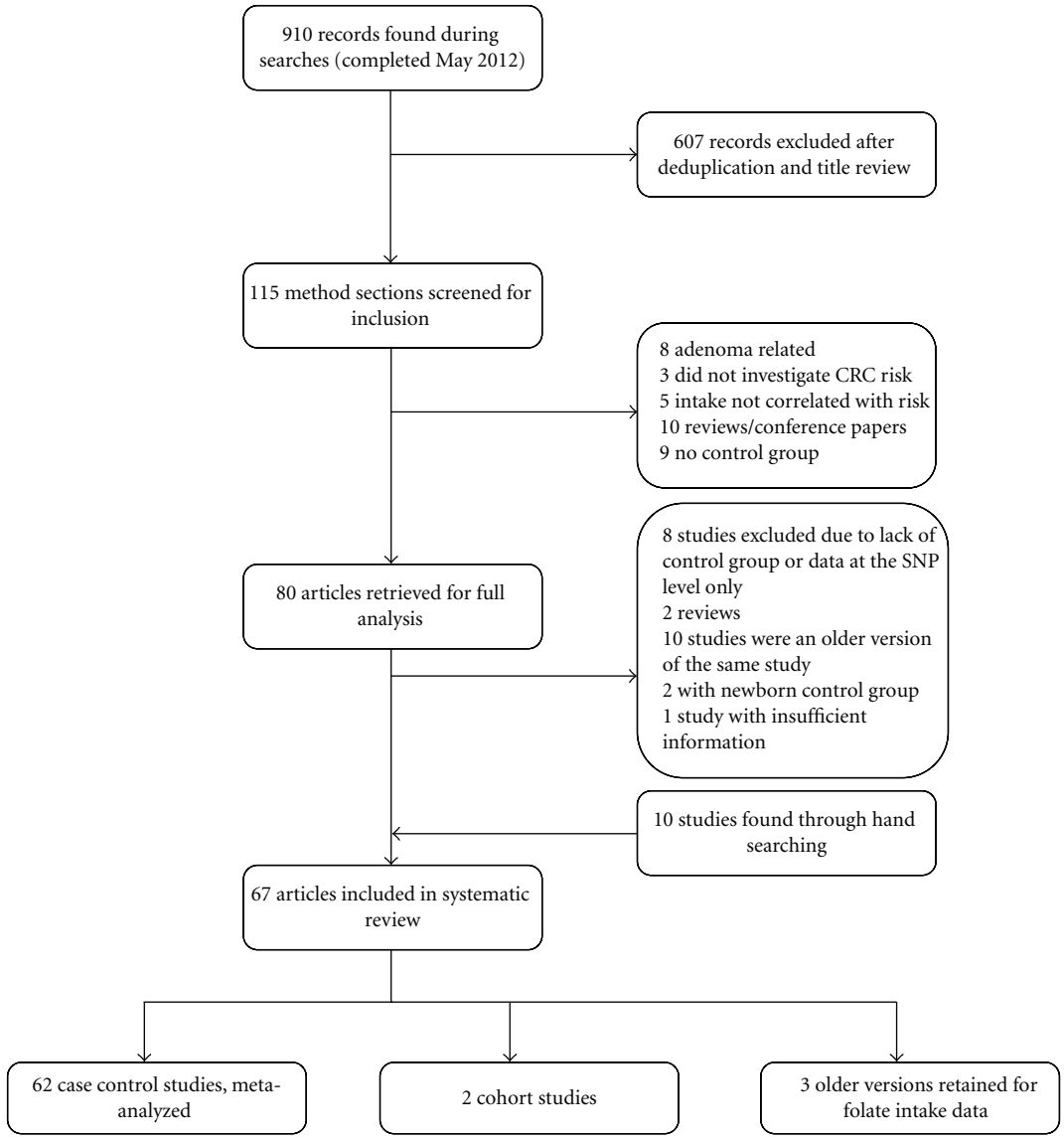


Figure 1: Search strategy flow chart.

Two studies, Wang et al and Promthet et al., did not have any case participants with a TT genotype [48, 69].

3.1.1. Subgroup Analysis. Subgroup analysis was performed on sex. The pooled summary risk estimate of the studies reporting on sex for *677*CT versus *677*CC was 1.04 (95% CI 0.94–1.16), $\chi^2 = 14.28, \text{df} = 10, P = 0.16, I^2 = 30\%$ and *677*TT versus *677*CC was 0.87 (95% CI 0.75–1.01), $\chi^2 = 14.01$, df = 10, $P = 0.17$, $I^2 = 29\%$ with heterogeneity (Table 2). The summary risk estimates for CRC risk between *677*CT versus *677*CC for men only were 1.12 (95% CI 0.94–1.34), $\chi^2 = 18.68, \text{df} = 8, P = 0.02, I^2 = 57\%$ with significant heterogeneity (Table 2) and for women only 0.98 (95% CI 0.85–1.12), $\chi^2 = 7.63$, df = 7, $P = 0.37$, $I^2 = 8\%$ (Table 2). The summary risk estimates for *677*TT versus *677*CC for men were 0.87 (95% CI 0.74–1.02), $\chi^2 = 8.36, \text{df} = 8, P = 0.40, I^2 = 4\%$ (Table 2) and for women only were 0.92 (95% CI 0.65–1.31), $\chi^2 = 20.74$, df = 7, $P = 0.004$, $I^2 = 66\%$ with significant heterogeneity (Table 2).

Separate estimates for colon cancer and rectal cancer were also evaluated. For the summary risk estimates related to colon or rectal cancer, only those studies that reported separate results for either colon or rectal cancer were included. The pooled summary risk estimate of the studies reporting on either colon or rectal cancer only for *677*CT versus *677*CC was 1.01 (95% CI 0.95–1.08) $\chi^2 = 23.65$, df = 26, $P = 0.60$, $I^2 = 0\%$ and *677*TT versus *677*CC was 0.80 (95% CI 0.71–0.89) $\chi^2 = 31.45, \text{df} = 23, P = 0.11, I^2 = 27\%$ with some heterogeneity evident (Table 2). The summary risk estimates for *677*CT versus *677*CC colon cancer only were 1.01 (95% CI 0.93–1.10), $\chi^2 = 11.23, \text{df} = 15, P = 0.74$, $I^2 = 0\%$ (Table 2) and *677*TT versus *677*CC colon cancer only 0.76 (95% CI 0.64–0.91 $\chi^2 = 22.79, \text{df} = 13, P = 0.03$, $I^2 = 43\%$ (Table 2). The summary risk estimates for *677*CT

Study or subgroup	Log (odds ratio)	SE	Weight	Odds ratio IV, random, 95% CI
Park CRC	−0.065	0.19	1.6%	0.94 [0.65, 1.36]
Naghibalhossaini CRC	1.03	0.231	1.3%	2.80 [1.78, 4.41]
Matsuo CRC	0.262	0.245	1.3%	1.30 [0.80, 2.10]
Otani CRC	−0.288	0.281	1.1%	0.75 [0.43, 1.30]
Battistelli CRC	−0.315	0.332	0.9%	0.73 [0.38, 1.40]
Cui CRC	−0.08	0.081	2.4%	0.92 [0.79, 1.08]
Toffoli colon	−0.079	0.192	1.6%	0.92 [0.63, 1.35]
Cao CRC	−0.062	0.173	1.7%	0.94 [0.67, 1.32]
Fernandez-Peralta CRC	−0.673	0.272	1.1%	0.51 [0.30, 0.87]
Jin CRC	−0.315	0.134	2.0%	0.73 [0.56, 0.95]
Abuli CRC	0.255	0.138	2.0%	1.29 [0.98, 1.69]
Miao CRC	0.077	0.201	1.5%	1.08 [0.73, 1.60]
Delgato-Enciso CRC	0.605	0.373	0.7%	1.83 [0.88, 3.80]
Yin CRC	−0.117	0.117	2.2%	0.89 [0.71, 1.12]
Zhang CRC	0.247	0.207	1.5%	1.28 [0.85, 1.92]
Shannon CRC	−0.284	0.113	2.2%	0.75 [0.60, 0.94]
Lightfoot CRC	−0.186	0.13	2.1%	0.83 [0.64, 1.07]
Haghighi CRC	−0.994	0.229	1.4%	0.37 [0.24, 0.58]
Plaschke CRC	0.247	0.185	1.6%	1.28 [0.89, 1.84]
Kury CRC	−0.073	0.095	2.3%	0.93 [0.77, 1.12]
Pardini CRC	−0.041	0.101	2.3%	0.96 [0.79, 1.17]
Eussen CRC	−0.592	0.07	2.5%	0.55 [0.48, 0.63]
Zeybek CRC	0.39	0.352	0.8%	1.48 [0.74, 2.94]
Matsuo (2) CRC	−0.128	0.123	2.1%	0.88 [0.69, 1.12]
Le Marchand CRC	0	0.106	2.2%	1.00 [0.81, 1.23]
Landi CRC	−0.03	0.176	1.7%	0.97 [0.69, 1.37]
Theodoratou CRC	−0.073	0.108	2.2%	0.93 [0.75, 1.15]
Chang CRC	0.068	0.215	1.4%	1.07 [0.70, 1.63]
Hubner CRC	0	0.071	2.5%	1.00 [0.87, 1.15]
Kim J CRC	0.02	0.124	2.1%	1.02 [0.80, 1.30]
Curtin colon	−0.039	0.084	2.4%	0.96 [0.82, 1.13]
Jokic colon	0.021	0.172	1.7%	1.02 [0.73, 1.43]
Jiang Q CRC	0.07	0.227	1.4%	1.07 [0.69, 1.67]
Lima CRC	0.278	0.258	1.2%	1.32 [0.80, 2.19]
Guimaraes CRC	0.824	0.438	0.6%	2.28 [0.97, 5.38]
Ulvik CRC	0.01	0.066	2.5%	1.01 [0.89, 1.15]
lacopetta dist CRC	−0.105	0.107	2.2%	0.90 [0.73, 1.11]
lacopetta prox CRC	0.27	0.145	1.9%	1.31 [0.99, 1.74]
Zhu CRC	0.549	0.321	0.9%	1.73 [0.92, 3.25]
Murtaugh rectal	−0.083	0.109	2.2%	0.92 [0.74, 1.14]
Komlosi colon	0.077	0.14	2.0%	1.08 [0.82, 1.42]
Komlosi rectal	0.336	0.139	2.0%	1.40 [1.07, 1.84]
Lee HPFS CRC	0.049	0.208	1.5%	1.05 [0.70, 1.58]
Lee NHS CRC	−0.117	0.189	1.6%	0.89 [0.61, 1.29]
Kim JW CRC	−0.337	0.426	0.6%	0.71 [0.31, 1.65]
Karpinski CRC	0.542	0.139	2.0%	1.72 [1.31, 2.26]
Van Quelpen CRC	−0.094	0.182	1.7%	0.91 [0.64, 1.30]
Le Marchand CRC	−0.223	0.162	1.8%	0.80 [0.58, 1.10]
Mokarram colon	0.478	0.292	1.0%	1.61 [0.91, 2.86]
Pufulete CRC	0.039	0.699	0.3%	1.04 [0.26, 4.09]
Lee PHS CRC	0.3	0.19	1.6%	1.35 [0.93, 1.96]
Wettergern CRC	0.381	0.202	1.5%	1.46 [0.99, 2.17]
Gallegos-Arreola CRC	−0.276	0.214	1.5%	0.76 [0.50, 1.15]
Keku colon	0.095	0.123	2.1%	1.10 [0.86, 1.40]
El Awady CRC	1.095	0.443	0.6%	2.99 [1.25, 7.12]
Osian CRC	0.451	0.376	0.7%	1.57 [0.75, 3.28]
Promthet colon	−0.329	0.309	1.0%	0.72 [0.39, 1.32]
Chandy CRC	0.166	0.345	0.8%	1.18 [0.60, 2.32]
Jiang colon	0.392	0.323	0.9%	1.48 [0.79, 2.79]
Jiang rectal	−0.223	0.289	1.0%	0.80 [0.45, 1.41]
Sameer CRC	0.307	0.344	0.8%	1.36 [0.69, 2.67]
Wang CRC	0.199	0.275	1.1%	1.22 [0.71, 2.09]
Sharp CRC	−0.2613	0.209	1.5%	0.77 [0.51, 1.16]
Prasad CRC	0.854	0.426	0.6%	2.35 [1.02, 5.41]
Total (95% CI)			**100.0%**	**1.02 [0.95, 1.10]**

Heterogeneity: $\tau^2 = 0.05$; $\chi^2 = 210.34$, df $= 63$ ($P < 0.00001$); $I^2 = 70\%$
Test for overall effect: $Z = 0.55$ ($P = 0.58$)

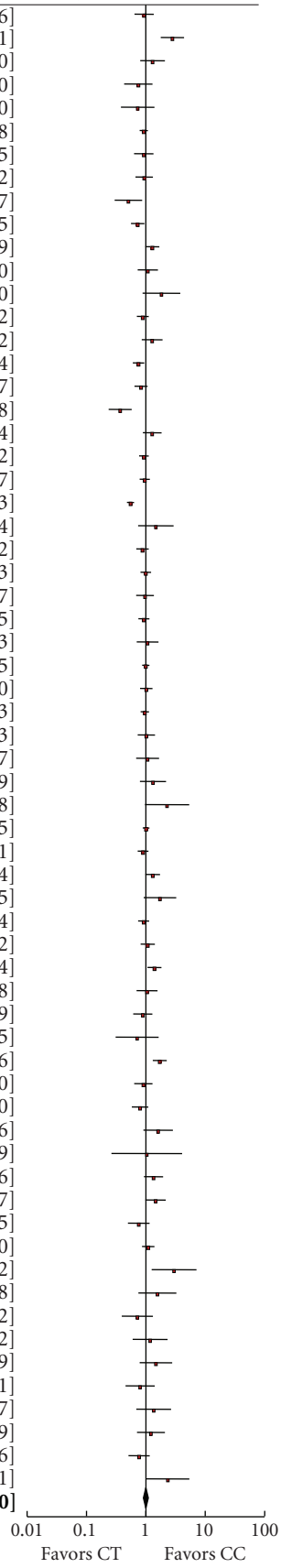


Figure 2: Forest plot of the risk of colorectal cancer for *MTHFR 677*CT versus CC.

Study or subgroup	Log (odds ratio)	SE	Weight	Odds ratio IV, random, 95% CI
Gallegos-Arreola CRC	0.571	0.254	1.8%	1.77 [1.08, 2.91]
Kim JW CRC	−1.58	0.549	0.6%	0.21 [0.07, 0.60]
Haghighi CRC	−0.673	0.248	1.8%	0.51 [0.31, 0.83]
Battistelli CRC	0.293	0.399	1.0%	1 .34 [0.61, 2.93]
Otani CRC	−0.236	0.327	1.3%	0.79 [0.42, 1.50]
Kim J CRC	−0.511	0.159	2.6%	0.60 [0.44, 0.82]
Zhang CRC	0.095	0.243	1.9%	1.10 [0.68, 1.77]
Delgato-Enciso CRC	0.473	0.443	0.9%	1.60 [0.67, 3.82]
Miao CRC	0.525	0.235	1.9%	1.69 [1.07, 2.68]
Jin CRC	−0.755	0.188	2.3%	0.47 [0.33, 0.68]
Toffoli colon	−0.502	0.259	1.8%	0.61 [0.36, 1.01]
Landi CRC	−0.117	0.224	2.0%	0.89 [0.57, 1.38]
Jiang Q CRC	−0.476	0.332	1.3%	0.62 [0.32, 1.19]
Jiang colon	−1.514	0.762	0.3%	0.22 [0.05, 0.98]
Jiang rectal	−0.198	0.378	1.1%	0.82 [0.39, 1.72]
Cao CRC	−0.094	0.115	3.1%	0.91 [0.73, 1.14]
Cui CRC	−0.233	0.111	3.1%	0.79 [0.64, 0.98]
Matsuo CRC	0.191	0.336	1.3%	1.21 [0.63, 2.34]
Yin CRC	−0.446	0.168	2.5%	0.64 [0.46, 0.89]
Park CRC	−0.205	0.268	1.7%	0.81 [0.48, 1.38]
Lee HPFS CRC	−0.434	0.315	1.4%	0.65 [0.35, 1.20]
Le Marchand CRC	−0.371	0.168	2.5%	0.69 [0.50, 0.96]
Lee PHS CRC	−0.625	0.319	1.4%	0.54 [0.29, 1.00]
Matsuo (2) CRC	−0.274	0.22	2.1%	0.76 [0.49, 1.17]
Abuli CRC	0.157	0.197	2.3%	1.17 [0.80, 1.72]
Lee NHS CRC	−0.261	0.298	1.5%	0.77 [0.43, 1.38]
Kury CRC	−0.051	0.14	2.8%	0.95 [0.72, 1.25]
Komlosi colon	0.174	0.205	2.2%	1.19 [0.80, 1.78]
Komlosi rectal	0.131	0.213	2.1%	1.14 [0.75, 1.73]
Sharp CRC	−0.478	0.354	1.2%	0.62 [0.31, 1.24]
Le Marchand CRC	−0.357	0.182	2.4%	0.70 [0.49, 1.00]
Chandy CRC	−0.151	1.421	0.1%	0.86 [0.05, 1 3.93]
Mokarram colon	−0.827	0.532	0.6%	0.44 [0.15, 1.24]
Hubner CRC	−0.139	0.106	3.1%	0.87 [0.71, 1.07]
Murtaugh rectal	−0.186	0.18	2.4%	0.83 [0.58, 1.18]
Eussen CRC	0.021	0.114	3.1%	1.02 [0.82, 1.28]
Theodoratou CRC	0.01	0.17	2.5%	1.01 [0.72, 1.41]
Curtin colon	−0.299	0.142	2.8%	0.74 [0.56, 0.98]
Plaschke CRC	0.122	0.294	1.5%	1.13 [0.63, 2.01]
Zeybek CRC	0.506	0.53	0.7%	1.66 [0.59, 4.69]
lacopetta prox CRC	0.207	0.232	2.0%	1.23 [0.78, 1.94]
lacopetta dist CRC	−0.261	0.191	2.3%	0.77 [0.53, 1.12]
Lima CRC	0.588	0.383	1.1%	1.80 [0.85, 3.81]
Karpinski CRC	−0.139	0.489	0.7%	0.87 [0.33, 2.27]
Zhu CRC	0.93	0.471	0.8%	2.53 [1.01, 6.38]
Pardini CRC	−0.545	0.207	2.2%	0.58 [0.39, 0.87]
Ulvik CRC	−0.315	0.118	3.0%	0.73 [0.58, 0.92]
Shannon CRC	0.032	0.181	2.4%	1.03 [0.72, 1.47]
Jokic colon	0.123	0.287	1.6%	1.13 [0.64, 1.98]
Guimaraes CRC	0.445	0.438	0.9%	1.56 [0.66, 3.68]
Fernandez-Peralta CRC	−2.813	0.854	0.3%	0.06 [0.01, 0.32]
Van Guelpen CRC	−0.892	0.372	1.1%	0.41 [0.20, 0.85]
Wettergern CRC	0.395	0.338	1.3%	1.48 [0.77, 2.88]
Lightfoot CRC	0.207	0.219	2.1%	1.23 [0.80, 1.89]
Chang CRC	0.482	0.357	1.2%	1.62 [0.80, 3.26]
Pufulete CRC	1.788	0.952	0.2%	5.98 [0.93, 38.62]
Sameer CRC	0.425	0.471	0.8%	1.53 [0.61, 3.85]
Keku colon	−0.223	0.286	1.6%	0.80 [0.46, 1.40]
El Awady CRC	0.451	0.919	0.2%	1.57 [0.26, 9.51]
Naghibalhossaini CRC	−0.357	0.509	0.7%	0.70 [0.26, 1.90]
Osian CRC	0.756	0.73	0.4%	2.13 [0.51, 8.91]
Prasad CRC	0.854	1.419	0.1%	2.35 [0.15, 37.91]
Total (95% lC)			**100.0%**	**0.88 [0.80,0.96]**

Heterogeneity: $\tau^2 = 0.06$; $\chi^2 = 132.66$, df $= 61$ ($P < 0.00001$); $I^2 = 54\%$
Test for overall effect: $Z = 2.78$ ($P = 0.005$)

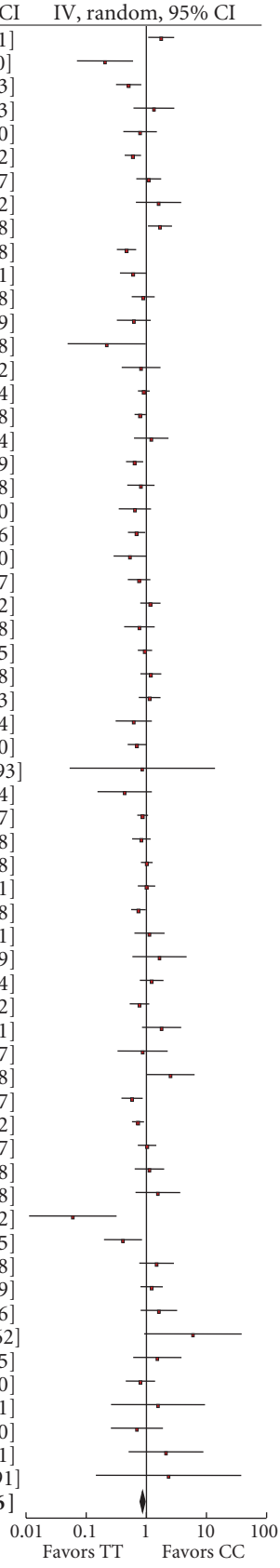


Figure 3: Forest plot of the risk of colorectal cancer for *MTHFR 677*TT versus CC.

Table 2: Subgroup analysis for the *MTHFR C677T* polymorphism.

	Number of studies	Number of participants in case/control		Summary effect estimate	95% CI	Tests for heterogeneity	
		CC genotype	Comparator genotype				
Subgroup by sex							
Pooled studies for sex							I^2 (%)
CT versus CC	11	1,650/1,833	1,420/1,523	1.04	0.94–1.16	$\chi^2 = 14.28$, df = 10 ($P = 0.16$)	30
TT versus CC	11	1,650/1,833	326/425	0.87	0.75–1.01	$\chi^2 = 14.01$, df = 10 ($P = 0.17$)	29
Men							
CT versus CC	9	1,257/1,436§	1,081/1,199§	1.12	0.94–1.34	$\chi^2 = 18.68$, df = 8 ($P = 0.02$)	57
TT versus CC	9	1,257/1,436§	271/346§	0.87	0.74–1.02	$\chi^2 = 8.36$, df = 8 ($P = 0.40$)	4
Women							
CT versus CC	8	755/897§	627/773§	0.98	0.85–1.12	$\chi^2 = 7.63$, df = 7 ($P = 0.37$)	8
TT versus CC	8	755/897§	162/217§	0.92	0.65–1.31	$\chi^2 = 20.74$, df = 7 ($P = 0.004$)	66
Subgroup by cancer type							
Pooled studies							
CT versus CC	27	3,735/6,767	3,403/6,307	1.01	0.95–1.08	$\chi^2 = 23.65$, df = 26 ($P = 0.60$)	0
TT versus CC	24*	3,735/6,767	886/2,117	**0.80**	0.71–0.89	$\chi^2 = 31.45$, df = 23 ($P = 0.11$)	27
Colon cancer studies							
CT versus CC	16	2,096/4,463	1,933/4,090	1.01	0.93–1.10	$\chi^2 = 11.23$, df = 15 ($P = 0.74$)	0
TT versus CC	14**	2,096/4,463	452/1,352	**0.76**	0.64–0.91	$\chi^2 = 22.79$, df = 13 ($P = 0.04$)	43
Rectal cancer studies							
CT versus CC	11	1,639/3,291	1,470/2,996	1.10	0.92–1.31	$\chi^2 = 27.95$, df = 10 ($P = 0.002$)	64
TT versus CC	10	1,639/3,291	386/1,020	**0.82**	0.72–0.94	$\chi^2 = 8.38$, df = 9 ($P = 0.50$)	0
Subgroup by location							
Asian countries							
CT versus CC	22	2,640/3,401	2,985/3,903	0.98	0.89–1.06	$\chi^2 = 23.98$, df = 21 ($P = 0.29$)	12
TT versus CC	20**	2,640/3,401	1,001/1,565	0.83	0.69–1.01	$\chi^2 = 49.66$, df = 19 ($P = 0.0001$)	62
European countries							
CT versus CC	22	5,480/6,960	5,374/6,857	1.00	0.87–1.13	$\chi^2 = 109.92$, df = 21 ($P < 0.00001$)	81
TT versus CC	22	5,480/6,960	1,294/1,793	0.92	0.80–1.06	$\chi^2 = 43.74$, df = 21 ($P = 0.003$)	52
USA							
CT versus CC	8	2,011/3,355	1,932/2,997	0.98	0.90–1.07	$\chi^2 = 6.07$, df = 7 ($P = 0.53$)	0
TT versus CC	8	2,011/3,355	436/1,055	**0.73**	0.63–0.84	$\chi^2 = 1.91$, df = 7 ($P = 0.96$)	0
Middle Eastern countries							
CT versus CC	5	277/374	274/302	1.46	0.62–3.46	$\chi^2 = 45.30$, df = 4 ($P < 0.00001$)	91
TT versus CC	5	277/374	72/105	0.69	0.42–1.13	$\chi^2 = 5.56$, df = 4 ($P = 0.23$)	28
Subgroup by control							
Healthy person controls							
CT versus CC	45	8,706/12,958	8,043/12,044	1.02	0.94–1.11	$\chi^2 = 154.26$, df = 44 ($P < 0.00001$)	71
TT versus CC	43**	8,706/12,958	2,136/3,636	0.90	0.81–1.00	$\chi^2 = 88.37$, df = 42 ($P = 0.0001$)	52
Hospital patient controls							
CT versus CC	16	2,418/2,863	2,932/3,619	0.93	0.83–1.05	$\chi^2 = 27.35$, df = 15 ($P = 0.03$)	45
TT versus CC	16	2,418/2,863	939/1,254	0.82	0.68–1.00	$\chi^2 = 36.07$, df = 15 ($P = 0.002$)	58

§Not all studies reported both case and control numbers.
*There were two studies without TT genotype information, one study with rectal cancer data, and two studies with colon cancer data.
**There were two studies that had 0 people for the TT genotype.
CRC: colorectal cancer.

versus *677*CC rectal cancer only were 1.10 (95% CI 0.92–1.31), $\chi^2 = 27.95$, df = 10, $P = 0.002$, $I^2 = 64\%$ (Table 2) and *677*TT versus *677*CC rectal cancer only 0.82 (95% CI 0.72–0.94), $\chi^2 = 8.38$, df = 9, $P = 0.50$, $I^2 = 0\%$ (Table 2).

3.1.2. Sensitivity Analysis. In an attempt to identify the studies contributing to the heterogeneity in the genotype summary risk effect results, sensitivity analysis was performed according the sequential algorithm proposed by Patsopoulos and colleagues [96]. This method involves sequentially dropping one study from the meta-analysis to determine the impact on the I^2 statistic with the objective of identifying the study or studies that will reduce the I^2 below a set threshold. Using this method, we were not successful in reducing the heterogeneity below the threshold value of an I^2 value of less than 25%, which would have suggested that there was minimal heterogeneity in the results.

Given that the typical diets of Asian cultures can be substantially different from that of Europe and North America, separate analyses were conducted including just the studies in the Asian locations (China, India, Japan, South Korea, and Taiwan), separate from the European locations (Czech Republic, Croatia, European EPIC study, France, Germany, Hungary, Italy, Norway, Poland, Romania, Spain, Sweden, and United Kingdom), USA, and Middle East (Egypt, Iran, and Turkey) (Table 2). The protective effect of the *677*TT genotype was sustained in each geography; however, only in the USA was the risk reduction significant with no heterogeneity.

A further analysis was performed by comparing the results based on the source of controls: either hospital patients or healthy persons. The heterogeneity was sustained (Table 2).

3.1.3. Publication Bias. Publication bias was assessed using three different tests: Begg and Mazumdar's rank correlation test**,** Egger's linear regression, and the Trim and Fill methods. For the *MTHFR 677*CT genotype there may be some evidence for publication bias. The Begg and Mazumdar test returned a P value = 0.03, Egger's a P value = 0.005, and Trim and Fill found that an additional 12 studies would be necessary to form a symmetrical funnel plot. Whereas, for the *MTHFR 677*TT genotype, the Begg and Mazumdar test returned a P value = 0.33, Egger's a P value = 0.38, and Trim and Fill found that additional 4 studies would be necessary to form a symmetrical funnel plot, suggesting that publication bias may not be significant concern.

3.1.4. Correlation between Study Quality versus Results. There was no statistically significant difference found in the quality of the studies based on outcome (positive versus neutral versus negative) ($P = 0.310$).

3.2. Colorectal Cancer Risk and MTHFR A1298C Genotype. For the comparison of *1298*AC versus *1298*AA, the summary risk estimate was 1.03 (95% CI 0.96–1.10), $\chi^2 = 54.54$, df = 39, $P = 0.05$, $I^2 = 28\%$ with some heterogeneity (Figure 4). For the comparison of *1298*CC versus *1298*AA genotype, the summary risk estimate was 0.93 (95% CI 0.82–1.06), $\chi^2 = 62.14$, df = 38, $P = 0.008$, $I^2 = 39\%$ with heterogeneity (Figure 5).

3.2.1. Sensitivity Analysis. In an attempt to identify the studies contributing to the heterogeneity in the genotype summary risk effect results for *1298*CC, the previously described process for sensitivity analysis was performed. The resulting summary effects estimate for *1298*CC versus *1298*AA was 1.04 (95% CI 0.94–1.14) $\chi^2 = 32.17$, df = 32, $P = 0.46$, $I^2 = 1\%$ with no significant heterogeneity (data not shown). In this analysis, the studies contributing to the heterogeneity were conducted in Germany, India, and the USA [35, 37, 48, 54, 76].

3.2.2. Subgroup Analysis. There were an insufficient number of studies that reported CRC risk by sex; however, subgroups, by geography, and source of controls were performed.

Subgroup analysis by geography was performed for the *MTHFR A1298C* polymorphism according to the country groups previously described. There were an insufficient number of studies from the Middle East to include this location in the analysis. The subgroup analysis revealed that for European countries there was an associated, significant increased risk of CRC for those with the *1298*CC genotype, while Asian and USA studies suggest a significant associated decrease in risk (Table 3). This variability in the associated risk of the *1298*CC genotype by geography was also noted by Kono and Chen in their meta-analysis [95].

A further analysis was performed by comparing the results based on the source of controls; either hospital patients or healthy persons. For the CC variant, the healthy controls had a nonsignificant reduced risk associated with CRC versus hospital control, within some increase in heterogeneity (Table 3).

3.2.3. Publication Bias. The results of the statistical test for publication bias for the *MTHFR A1298C* polymorphisms suggest that publication bias may not be a concern. For *MTHFR 1298*AC, the Begg and Mazumdar test returned a P value = 0.24, Egger's a P value = 0.398, and Trim and Fill found that an additional 5 studies would be necessary to form a symmetrical funnel plot whereas, for the *1298*CC genotype, the Begg and Mazumdar test returned a P value = 0.88, Egger's a P value = 0.74, and Trim and Fill found that no additional studies would be necessary to form a symmetrical funnel plot.

3.3. Colorectal Cancer Risk and Combinations of the MTHFR C677T and A1298C Genotypes. The combinations of variants of the *MTHFR C677T* and *A1298C* genotypes are in linkage disequilibrium such that rarely are there individuals with the *677*TT/*1298*AC and *677*TT/*1298*CC combinations [95]. The results of the summary risk estimates for the remaining combinations are presented in Table 4. The combination of *677*TT/*1298*AA was associated with lowest risk of CRC with a summary risk estimate of 0.77 (95% CI

Study or subgroup	Log (odds ratio)	SE	Weight	Odds ratio IV, random, 95% CI
Chandy CRC	0.519	0.309	1.0%	1.68 [0.92, 3.08]
Promthet colon	0.405	0.267	1.3%	1.50 [0.89, 2.53]
El Awady CRC	0.191	0.458	0.5%	1.21 [0.49, 2.97]
Wang CRC	−0.478	0.201	2.1%	0.62 [0.42, 0.92]
Pardini CRC	0.058	0.104	5.1%	1.06 [0.86, 1.30]
Naghibalhossaini CRC	0.262	0.268	1.3%	1.30 [0.77, 2.20]
Theodoratou CRC	−0.186	0.105	5.0%	0.83 [0.68, 1.02]
Lightfoot CRC	−0.041	0.131	3.9%	0.96 [0.74, 1.24]
Murtaugh rectal	−0.151	0.116	4.5%	0.86 [0.68, 1.08]
Plaschke CRC	0.077	0.184	2.4%	1.08 [0.75, 1.55]
Toffoli colon	0.15	0.177	2.6%	1.16 [0.82, 1.64]
Fernandez-Peralta CRC	−0.274	0.241	1.6%	0.76 [0.47, 1.22]
Jokic colon	0.082	0.172	2.7%	1.09 [0.77, 1.52]
Eussen CRC	0.035	0.073	6.8%	1.04 [0.90, 1.19]
Lee HPFS CRC	0.1	0.205	2.1%	1.11 [0.74, 1.65]
Lee PHS CRC	0.068	0.179	2.5%	1.07 [0.75, 1.52]
Van Guelpen CRC	0.344	0.193	2.3%	1.41 [0.97, 2.06]
Sharp CRC	0.191	0.214	1.9%	1.21 [0.80, 1.84]
Kury CRC	0.182	0.097	5.4%	1.20 [0.99, 1.45]
Keku colon	−0.105	0.147	3.4%	0.90 [0.67, 1.20]
Landi CRC	0.01	0.162	2.9%	1.01 [0.74, 1.39]
Lee NHS CRC	0.415	0.197	2.2%	1.51 [1.03, 2.23]
Osian CRC	0.489	0.349	0.8%	1.63 [0.82, 3.23]
Le Marchand CRC	−0.105	0.102	5.2%	0.90 [0.74, 1.10]
Pufulete CRC	−0.598	0.71	0.2%	0.55 [0.14, 2.21]
Matsuo CRC	0.058	0.232	1.7%	1.06 [0.67, 1.67]
Chang CRC	0.215	0.22	1.8%	1.24 [0.81, 1.91]
Cao CRC	0.01	0.167	2.8%	1.01 [0.73, 1.40]
Yin CRC	0.068	0.115	4.6%	1.07 [0.85, 1.34]
Miao CRC	−3.912	1.398	0.1%	0.02 [0.00, 0.31]
Matsuo (2) CRC	−0.073	0.155	3.1%	0.93 [0.69, 1.26]
Lima CRC	−0.117	0.266	1.3%	0.89 [0.53, 1.50]
Jiang colon	0.058	0.324	0.9%	1.06 [0.56, 2.00]
Jiang Q CRC	−0.341	0.241	1.6%	0.71 [0.44, 1.14]
Jiang rectal	−0.654	0.339	0.9%	0.52 [0.27, 1.01]
Kim JW CRC	0.118	0.398	0.6%	1.13 [0.52, 2.45]
Zhang CRC	0.148	0.204	2.1%	1.16 [0.78, 1.73]
Otani CRC	0	0.271	1.3%	1.00 [0.59, 1.70]
Guimaraes CRC	0.476	0.282	1.2%	1.61 [0.93, 2.80]
Curtin colon	0.017	0.084	6.2%	1.02 [0.86, 1.20]
Total (95% lC)			**100.0**%	**1.03 [0.96, 1.10]**

Heterogeneity: $\tau^2 = 0.01$; $\chi^2 = 54.54$, df $= 39$ ($P = 0.05$); $I^2 = 28\%$
Test for overall effect: $Z = 0.82$ ($P = 0.41$)

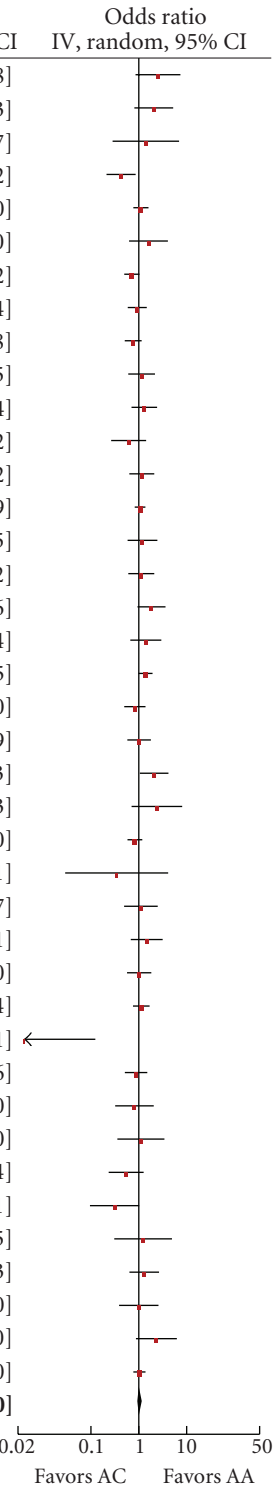


Figure 4: Forest plot of the risk of colorectal cancer for *MTHFR 1298*AC versus AA.

0.58–1.03), $\chi^2 = 19.00$, df $= 11$, $P = 0.06$, $I^2 = 42\%$ with significant heterogeneity.

3.4. Colorectal Cancer Risk, Comparison of High versus Low Folate Intake by Genotype. Of the articles that met our inclusion criteria, there were 10 studies that reported on CRC risk by "quantile" of folate intake for the *MTHFR C677T* polymorphism; however, an insufficient number of studies reported on the folate intake for the *A1298C* polymorphism to complete the analysis for this polymorphism. A food

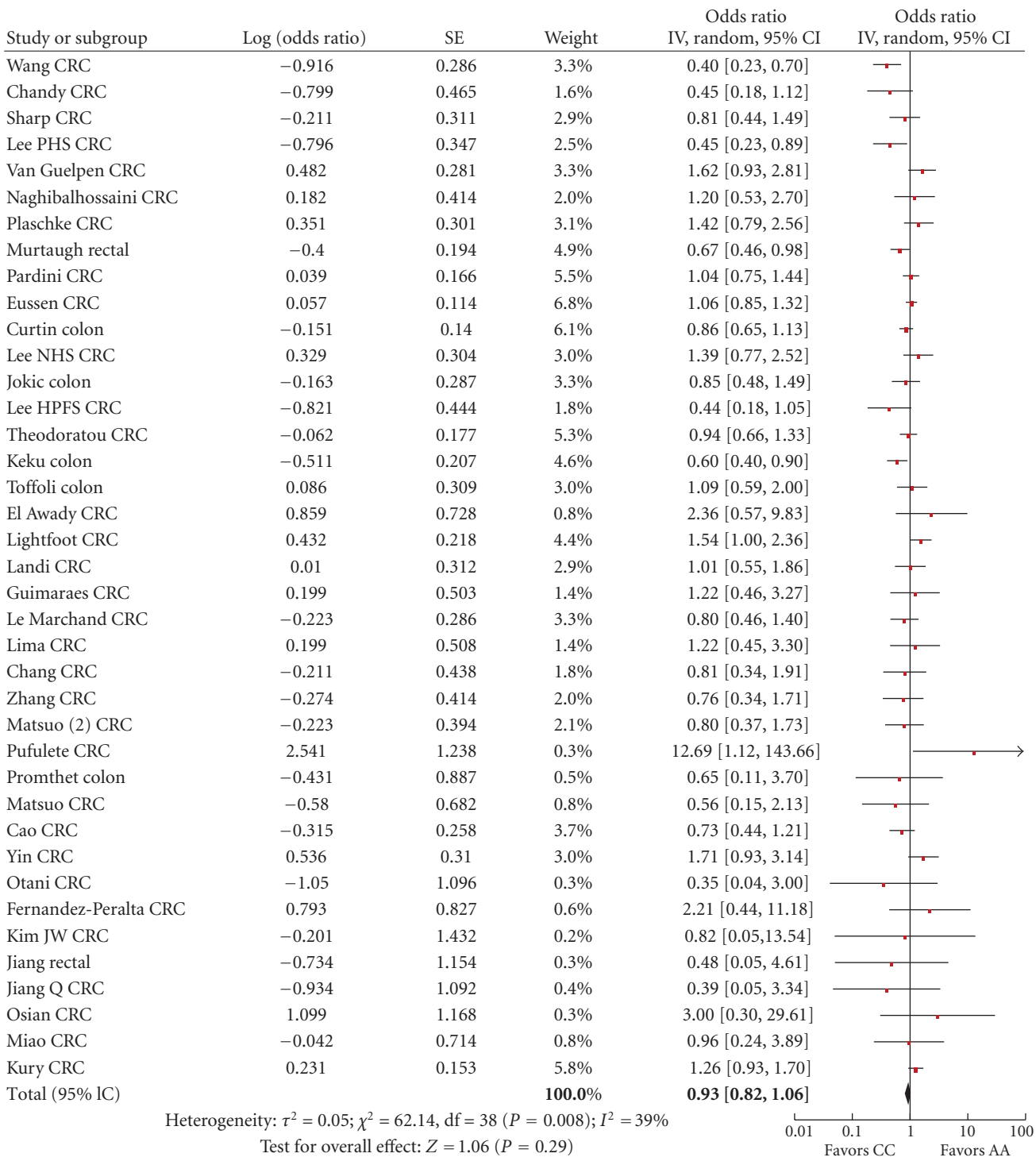


Figure 5: Forest plot of the risk of colorectal cancer for *MTHFR 1298*CC versus AA.

frequency questionnaire (FFQ) was the usual method used to collect dietary intake information. Dietary information was captured for one to two years preceding diagnosis, or for the control group, at the time of enrolment in the study. The range of dietary folate intake, defined as folate from food sources, for the lowest "quantile" ranged from a low of less than 115.6 to 406 mcg/day; the range for the highest was from 320 to 485 mcg/day or more (Table 5). The summary risk estimate for high versus low dietary folate intake for the *677*CC genotype was 0.76 (95% CI 0.62–0.94), $\chi^2 = 2.96$, df = 5, $P = 0.71$, $I^2 = 0\%$, for the *677*CT genotype 0.88 (95% CI 0.76–1.02), $\chi^2 = 1.44$, df = 2, $P = 0.49$,

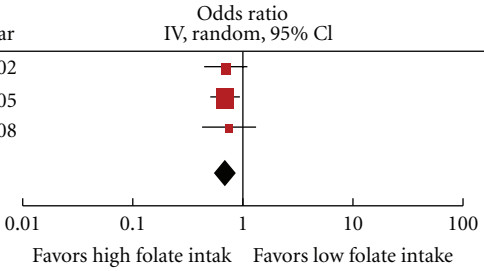
High versus low total folate intake

*MTHFR 677*CC

Study or subgroup	Log (odds ratiol)	SE	Weight	Odds ratio IV, random, 95% Cl	Year
Le Marchand CRC	−0.35667	0.230605	26.5%	0.70 [0.45, 1.10]	2002
Le Marchand CRC	−0.37106	0.157749	56.6%	0.69 [0.51, 0.94]	2005
Sharp CRC	−0.28768	0.288425	16.9%	0.75 [0.43, 1.32]	2008
Total (95% Cl)			**100.0%**	**0.70 [0.56, 0.89]**	

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 0.06$, df $= 2$ ($P = 0.97$); $I^2 = 0\%$

Test for overall effect: $Z = 2.98$ ($P = 0.003$)

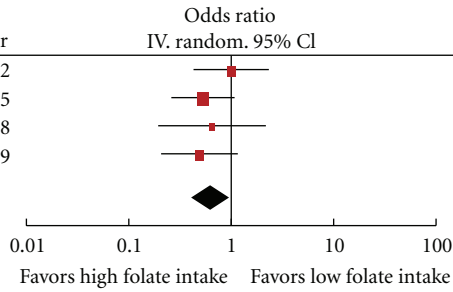


*MTHFR 677*TT

Study or subgroup	Log (odds ratio)	SE	Weight	Odds ratio IV, random, 95% Cl	Year
Le Marchand CRC	0	0.431565	25.9%	1.0 [0.43, 2.33]	2002
Le Marchand CRC	−0.63488	0.363183	36.5%	0.53 [0.26, 1.08]	2005
Lightfoot CRC	−0.43078	0.617402	12.6%	0.65 [0.19, 2.18]	2008
Haghighi CRC	−0.71335	0.439679	24.9%	0.49 [0.21, 1.16]	2009
Total (95% Cl)			**100%**	**0.63 [0.41, 0.97]**	

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 1.70$, df $= 3$ ($P = 0.64$); $I^2 = 0\%$

Test for overall effect: $Z = 2.11$ ($P = 0.03$)



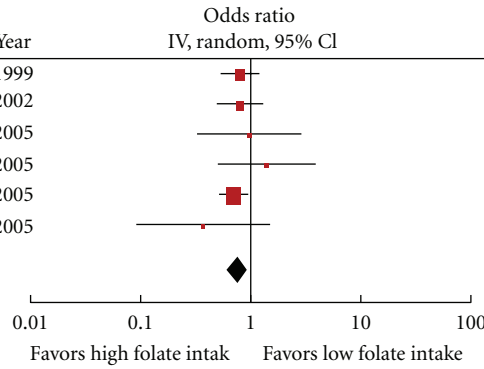
High versus low dietary folate intake

*MTHFR 677*CC

Study or subgroup	Log (odds ratio)	SE	Weight	Odds ratio IV, random, 95% Cl	Year
Slattery colon	−0.22314	0.20687	26.0%	0.80 [0.53, 1.20]	1999
Le Marchand CRC	−0.22314	0.247708	18.2%	0.80 [0.49, 1.30]	2002
Jiang Rectal	−0.03046	0.556998	3.6%	0.97 [0.33, 2.89]	2005
Otani CRC	0.336472	0.522706	4.1%	1.40 [0.50, 3.90]	2005
Le Marchand CRC	−0.35667	0.155807	45.9%	0.70 [0.52, 0.95]	2005
Jiang Colon	−0.99425	0.714142	2.2%	0.37 [0.09, 1.50]	2005
Total (95% Cl)			**100%**	**0.76 [0.62, 0.94]**	

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 2.96$, df $= 5$ ($P = 0.71$); $I^2 = 0\%$

Test for overall effect: $Z = 2.57$ ($P = 0.01$)

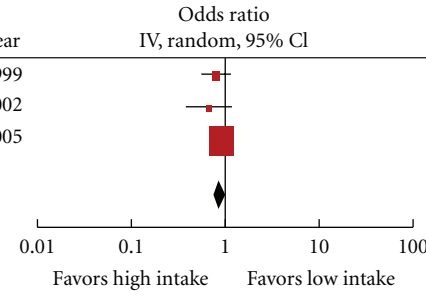


*MTHFR 677*CT

Study or subgroup	Log (odds ratio)	SE	Weight	Odds ratio IV, random, 95% Cl	Year
Slattery Colon	−0.22314	0.185156	15.8%	0.80 [0.56, 1.15]	1999
Le Marchand CRC	−0.40048	0.288771	6.5%	0.67 [0.38, 1.18]	2002
Le Marchand CRC	−0 .08338	0.08338	77.8%	0.92 [0.78, 1.08]	2005
Total (95% Cl)			**100.0%**	**0.88 [0.76, 1.02]**	

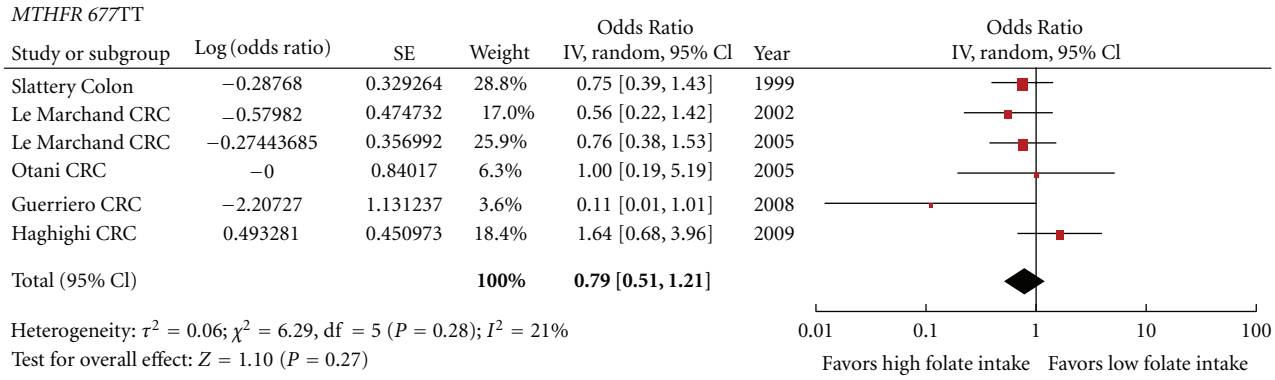
Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 1.44$, df $= 5$ ($P = 0.49$); $I^2 = 0\%$

Test for overall effect: $Z = 1.71$ ($P = 0.09$)



(a)

Figure 6: Continued.



(b)

Figure 6: Forest plot of the risk of colorectal cancer comparing high versus low folate intake within each *MTHFR C677T* polymorphism.

Table 3: Subgroup analysis for the *MTHFR A1298C* polymorphism.

	Number of studies	Number of participants in case/control		Summary effect estimate	95% CI	Tests for heterogeneity	
		AA genotype	Comparator genotype				
Subgroup by location							
Asian countries							I (%)
AC versus AA	15	1,727/3,047	991/1,615	0.99	0.84–1.16	$\chi^2 = 26.56$, df = 14 ($P = 0.02$)	47
CC versus AA	14*	1,727/3,047	116/178	**0.72**	0.55–0.93	$\chi^2 = 14.37$, df = 13 ($P = 0.35$)	10
European countries							
AC versus AA	14	2,971/4,119	2,404/3,746	1.05	0.97–1.14	$\chi^2 = 14.78$, df = 13 ($P = 0.32$)	12
CC versus AA	14	2,971/4,119	683/908	**1.14**	1.01–1.28	$\chi^2 = 13.13$, df = 13 ($P = 0.44$)	1
USA							
AC versus AA	7	1,678/2,694	1,365/2,244	0.99	0.88–1.11	$\chi^2 = 7.96$, df = 6 ($P = 0.24$)	25
CC versus AA	7	1,678/2,694	247/559	**0.73**	0.57–0.92	$\chi^2 = 10.20$, df = 6 ($P = 0.12$)	41
Subgroup by control							
Hospital controls							
AC versus AA	12	1,872/2,795	1,258/1,874	1.05	0.95–1.16	$\chi^2 = 4.42$, df = 11 ($P = 0.96$)	0
CC versus AA	12	1,872/2,795	232/311	1.12	0.88–1.42	$\chi^2 = 13.22$, df = 11 ($P = 0.28$)	17
Healthy controls							
AC versus AA	27	5,083/7,939	3,926/6,325	1.02	0.93–1.11	$\chi^2 = 48.87$, df = 26 ($P = 0.004$)	47
CC versus AA	26*	5,083/7,939	912/1,439	0.88	0.76–1.03	$\chi^2 = 45.33$, df = 25 ($P = 0.008$)	45

*There was one study that had no results for this genotype.

I^2 = 0% and the *677*TT genotype 0.78 (95% CI 0.53–1.13), χ^2 = 6.41, df = 6, P = 0.38, I^2 = 6% (Figure 6).

Total folate intake information was also reported in some studies. Total folate was defined as folate from dietary and supplemental sources. The lowest "quantile" ranged from less than 264 to 450 mcg/day and the higher "quantile" ranged from 348 to 1583 mcg/day or more (Table 5). The summary risk estimate for high versus low total folate intake for the *677*CC genotype was 0.70 (95% CI 0.56–0.89), χ^2 = 0.06, df = 2, P = 0.97, I^2 = 0% and the *677*TT genotype 0.63 (95% CI 0.41–0.97), χ^2 = 1.70, df = 3, P = 0.64, I^2 = 0% (Figure 6). Only two studies had information available for the *677*CT genotype; therefore, the summary risk estimate was not determined.

4. Discussion

The results of the analysis suggest that the homozygous variant genotype *MTHFR 677*TT confers a degree of protection against the development of CRC, affording an associated risk reduction of 12%. In contrast, the heterozygous genotype, *MTHFR 677*CT, was found to have the same risk as the genotype, *MTHFR 677*CC. These results are consistent with the previous meta-analysis completed in 2009 [94]. The thermolabile nature of *MTHFR 677*TT enzyme results in the reduced conversion of 5,10-methylene-tetrahydrofolate to 5-methyl-tetrahydrofolate, which acts as cofactor in the conversion of homocysteine to methionine, permitting a larger pool of 5,10-methylene-tetrahydrofolate

Table 4: Summary effect estimate results for the *MTHFR C677T* and *A1298C* polymorphism combinations.

Comparator genotype	Number of studies	Number of participants in case/control		Summary effect estimate	95% CI	Tests for heterogeneity	
		CC/AA genotype	Comparator genotype[§]				I (%)
CC/AC	12	609/775	677/870	0.96	0.82–1.11	$\chi^2 = 7.56$, df = 11 ($P = 0.75$)	0
CC/CC	12	609/775	180/312	0.90	0.64–1.27	$\chi^2 = 21.33$, df = 11 ($P = 0.03$)	48
CT/AA	12	609/775	753/912	0.99	0.86–1.15	$\chi^2 = 9.63$, df = 11 ($P = 0.56$)	0
CT/AC	12	609/775	491/678	1.06	0.79–1.44	$\chi^2 = 30.68$, df = 11 ($P = 0.001$)	64
CT/CC	5	609/775	18/36	1.40	0.33–6.03	$\chi^2 = 7.78$, df = 4 ($P = 0.10$)	49
TT/AA	12	609/775	311/465	0.77	0.58–1.03	$\chi^2 = 19.00$, df = 11 ($P = 0.06$)	42
TT/AC	4	609/775	11/17	N/a			
TT/CC	3	609/775	0/6	N/a			

[§] There was one study that did not report case control numbers for the combinations.

for thymidylate biosynthesis. This protective effect would suggest that preferential availability of folates to contribute pyrimidine synthesis, and therefore a reduction in uracil misincorporation and subsequent DNA breaks, could be important in the pathogenesis of CRC [32].

This reduced risk of CRC for the *677*TT genotype was not supported by all of the included studies. In several individual studies, the *677*TT genotype was associated with an increased risk of CRC [29–31]. The authors of these studies theorized that conditions of low folate intake, which is characteristic of the diet in these countries (Brazil, Mexico), may explain the increased risk found between the *677*TT genotype and CRC. This would appear to be substantiated by the reduced risk apparent in the summary risk estimated for *677*CC and *677*TT genotypes when comparing high versus low total folate intake (Figure 6) and would suggest that folate intake can alter the risk of CRC. Evidence for the alteration of disease through adequate folic acid intake has been found in other situations. For example, a maternal *MTHFR 677*TT genotype is associated with a higher risk of having an offspring with a neural tube defect [97]. Increased folic acid supplementation, periconceptionally and during the first trimester, has been found to reduce this risk [98].

Many of the studies incorporated both men and women into the case control groups. However, far fewer studies stratified their results based on sex. Of the eleven studies included in this subgroup analysis, representing over 7,000 case/control study participants, only one reported significant OR based on sex and genotype, which was contrary to the summary results in this meta-analysis (Table 2). Lightfoot et al. found that the men with the *677*CT genotype had a reduced risk of CRC, and women with the *677*TT genotype had an increased risk [58]. In the subgroup analysis on sex, the risk reduction of the *677*TT genotype and significant summary risk estimate for both sexes was no longer evident. This may represent lack of statistical power; it is possible that more studies are necessary to determine whether there may be a gender bias favoring one sex over another regarding the protective nature of the *677*TT genotype.

The *A1298C* polymorphisms would not appear to be associated with any substantial reduction in the associated risk of CRC. However, subgroup analysis did reveal some variability in the associated risk for the *1298*CC genotype, with lower risks associated with Asian and USA studies. What might be contributing to these geographical differences is unclear. Perhaps, as with the subgroup analysis by sex, additional studies with larger numbers of participants with this genotype are necessary to more clearly understand the relationship.

Many of the studies included in the high versus low folate intake meta-analysis compared the risk of CRC using the *677*CC or *677*CC/CT genotype and low folate intake as the reference group for the calculation of the odds ratio in other genotypes and folate intake "quantiles." Generally, the findings of these studies were that high folate intake and the *677*TT genotype were associated with a nonsignificant reduction in CRC risk versus low folate intake. This is the first study to perform a meta-analysis of the risk of CRC comparing high versus low folate intake within a genotype. The meta-analysis findings for the homozygous genotypes (*677*CC and *677*TT) indicate that there is greater risk reduction with higher levels of folate intake. The upper range of high folate intake reported in the studies was, generally, over the Institute of Medicine's (IOM) recommended daily intake (RDI) of 400 mcg/day and in one case over 1 mg/day [23, 99]. There were no clear boundaries in the definition of low folate intake versus high folate intake in this analysis as there was overlap in the ranges in daily folate amounts that defined the lowest folate intake versus the highest intake. This does prevent generalizing an amount of folate intake for each genotype that may be related to reducing colorectal cancer risk, which is a limitation of this analysis. Further, there is insufficient data to verify the shape (linear versus nonlinear) of the dose effect curve. More studies at this level of detail are necessary to provide further insight into the shape of the dose effect curve for folate and its associated impact on the risk of colorectal cancer.

The available studies used food frequency questionnaires (FFQs) or an adapted Coronary Artery Risk Development

Table 5: Case Control Studies: comparison of high versus low folate intake.

Study	Country	Year	Study design	Population of controls	Recruitment period	Cancer	Gender	Number of quantiles	Dietary folate (mcg/day)	Total folate (mcg/day)	Adjustments
Chen et al. [90]	USA	1996	Nested case control	Healthy persons	1986–1994	CRC	Men	3	<317 versus >461		Adjusted for age, family history, and intake of folate, methionine, and alcohol
Slattery et al. [91]	USA	1999	Matched case control	Healthy persons	1991–1994	Colon	Both	3	<126 versus >197 per 1000 kcals		Adjusted for age, BMI, physical activity, energy intake, dietary fiber, and smoking
Le Marchand et al. [23]	USA	2002	Matched case control	Healthy persons	1994–1998	CRC	Both	3	<278 versus >372	<336 versus >1583	Adjusted for age, gender, ethnicity, pack years of cigarette smoking, lifetime recreational physical activity, lifetime aspirin use, BMI 5 years ago, years of schooling, and intakes of nonstarch polysaccharides from vegetables and calcium from foods and supplements
Jiang et al. [42]	China	2005	Nested case control	Healthy persons	1989-1990	Colon rectal	Both	4	<115.6 versus >172 per 1000 kcals		Adjusted for sex, age, methionine, smoking status drinking status, and zinc
Le Marchand et al. [46]	USA (Hawaii and California)	2005	Nested case control	Healthy persons	1993–1996	CRC	Both	3	<253 versus >412	<322 versus >590	Adjusted for age, gender, and ethnicity
Otani et al. [45]	Japan	2005	Matched case control	Hospital patients	1998–2002	CRC	Both	3	<343 versus >485		Matching factors and adjusted for smoking, alcohol consumption, BMI, and total dietary fiber intake
Lightfoot et al. [58]	United Kingdom	2008	Matched case control	Hospital patients	1997–2000	CRC	Both	3		<267 versus >397	Adjusted for gender, and age
Sharp et al. [60]	United Kingdom	2008	Matched case control	Healthy persons	1998–2000	CRC	Both	4		<263.9 versus >348.6	Adjusted for age, gender and total energy intake.
Guerreiro et al. [31]	Portugal	2008	Case control	Healthy persons	Not reported	CRC	Both	2	≤406.7>		Adjusted for age, gender and CRC history
Haghighi et al. [22]	Iran	2009	Case control	Hospital patients	2004–2007	CRC	Both	2	≤320>	≤450>	Not reported

BMI: body mass index, CRC: colorectal cancer.

in Young Adults (CARDIAs) dietary history questionnaire to capture the food eaten on a regular basis; however, it is possible that not all of the folate food sources were captured thereby underestimating intake. Furthermore, tools such as the FFQ in case control studies are subject to recall bias since dietary intake was surveyed after a diagnosis of CRC. These two factors could lead to some under- or overreporting of folate intake resulting in misclassification of participants into their respective "quantiles." While mandatory folate fortification was implemented in the USA in 1998, none of the studies included in the meta-analysis on folate intake were conducted during times of folate fortification. Interestingly, a recent large observational study conducted in USA, after the mandated folate fortification period, found that higher folate intake levels were associated with a protective effect against CRC [100].

The studies included in the meta-analysis were conducted in twenty-five different countries. This is potentially both a strength and weakness of our analysis. Different countries represent different sources of folate and different food choice combinations, thus broadening the generalizability of our results. The potential weakness rests with the increased heterogeneity of some of the results. In the 2009 meta-analysis conducted by Taioli et al, their results indicate that in Asia the *677*TT genotype was afforded a significant risk reduction [94]. In our analysis, the *677*TT genotype is no longer significantly protective.

In conclusion, the results of this meta-analysis suggest that the *MTHFR 677*TT genotype is associated with a reduced risk of CRC. In addition, under conditions of high total folate intake, the associated risk of CRC is also reduced for both the *MTHFR 677* CC and TT genotypes.

Conflict of Interests

D. A. Kennedy is supported by a career development grant from Sickkids Foundation. G. Koren holds the Research Leadership for Better Pharmacotherapy during Pregnancy and Breastfeeding (Sickkids Hospital) and the Ivey Chair in Molecular Toxicology (University of Western Ontario). The Motherisk Program is conducting research supported by Duchesnay Inc. manufacturer of prenatal vitamins. These vitamins were not utilized in any of the studies included in this meta-analysis. The remaining authors have no financial interests to declare.

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