Review Article

Genetic polymorphisms of methylenetetrahydrofolate reductase and colorectal cancer and adenoma

Suminori Kono[1,3] and Kun Chen[2]

[1]Department of Preventive Medicine, Faculty of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan; and [2]Department of Epidemiology and Health Statistics, Zhejiang University School of Medicine, 353 Yan-an Road, Hangzhou 310031, China

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Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme regulating folate metabolism, which affects DNA methylation and synthesis. Two functional, common polymorphisms (*C677T* and *A1298C*) are known in the *MTHFR* gene. MTHFR activity is lowered in individuals with the *677TT* genotype and is somewhat reduced in those with the *1298CC* genotype. We reviewed the consistency of reported associations of these polymorphisms with colorectal cancer and adenoma with consideration of the effects of nutritional status. A total of 16 studies have addressed the association between *MTHFR C677T* polymorphism and colorectal cancer in 10 countries. Decreased risk of colorectal cancer associated with the *677TT* genotype has fairly consistently been observed, with few exceptions. This decrease was observable in people with either high or low folate status. Alteration in the thymidylate pool associated with MTHFR activity is postulated as an underlying mechanism. Studies on the *A1298C* polymorphism are limited, and their results are variable. Almost all of seven studies of colorectal adenoma have found no association between *C677T* polymorphism and adenoma, but the *677TT* genotype seems to be related to increased risk when folate status is poor. Reduced availability of methyl groups for DNA methylation might be more relevant to adenoma formation. Although the underlying mechanisms still remain to be clarified, epidemiological findings regarding *MTHFR C677T* polymorphism provide strong evidence that adequate folate status confers protection from colorectal cancer. (*Cancer Sci* 2005; *96*: 535–542)

Colorectal cancer is one of the most common cancers in the world, accounting for nearly 10% of new cases of all cancers.[1] The incidence of colorectal cancer varies substantially worldwide, with high rates in Western countries and low rates in African and Asian countries in general.[2] Japan has experienced a marked increase in the incidence of colorectal cancer, especially of colon cancer, and has recently been listed in the group of countries with the world's highest incidence rates.[3] It is thought that colorectal adenoma precedes the majority of colorectal cancers. The adenoma–carcinoma sequence model was originally based on pathological observations,[4] and has been strengthened by the observation of genetic alterations in the occurrence of adenoma and transition to carcinoma.[5] Over the past decade, the role of folate and genetic polymorphisms of enzymes in folate metabolism has attracted much interest in epidemiological research on colorectal cancer.[6,7]

Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme regulating folate metabolism, which is thought to influence DNA methylation and synthesis (Fig. 1).[8,9] MTHFR irreversibly converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which provides the methyl group to convert homocysteine to methionine, the precursor of *S*-adenosylmethionine (SAM). SAM is the universal methyl-group donor for methylation of a wide variety of biological substrates. It has been hypothesized that folate/methyl depletion may result in not only global genomic hypomethylation, but also aberrant methylation of CpG clusters in the promoters of tumor suppressor and DNA repair genes, probably via upregulation of DNA methyltransferase.[10–12] The substrate of MTHFR, 5,10-methylenetetrahydrofolate, is required for conversion of deoxyuridylate to thymidylate. Depletion of the thymidylate pool results in uracil misincorporation into DNA, leading to single- and double-strand breaks.[13–15]

Two common functional polymorphisms are known in the *MTHFR* gene. One is the *C677T* polymorphism, which results in an alanine-to-valine substitution at codon 222,[16] and the other is the *A1298C* polymorphism, which results in a substitution of glutamate with alanine at codon 429.[17] Individuals with the *677TT* genotype (variant homozygotes) have no more than 30% of normal enzyme activity, and heterozygotes (*CT*) have 65% of normal enzyme activity.[16] For the *MTHFR A1298C* polymorphism, enzyme activity is 40% lower in those with the *1298CC* genotype (variant homozygotes) than in those with the *1298AA* genotype.[17]

In this review, we evaluate the consistency of reported associations of the *MTHFR* polymorphisms with colorectal

[3]To whom correspondence should be addressed.
E-mail: skono@phealth.med.kyushu-u.ac.jp

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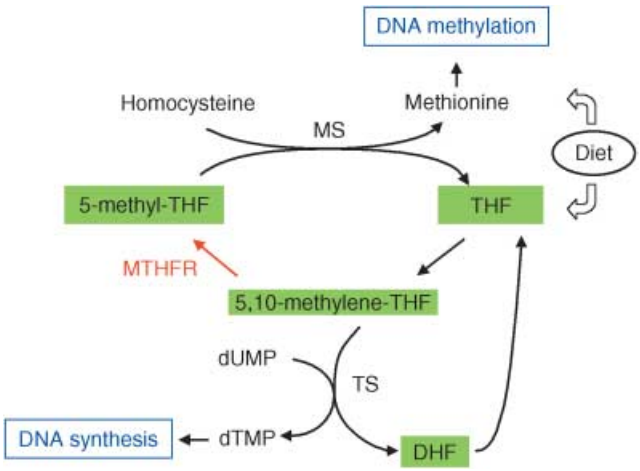

Fig. 1. Schematic presentation of folate metabolism in relation to DNA methylation and thymidylate synthesis. THF, tetrahydrofolate; MS, methionine synthase; TS, thymidylate synthase; dUMP, deoxyuridine monophosphate (deoxyuridylate); dTMP, deoxythymidine monophosphate (deoxythymidylate). The *MTHFR C677T* and *A1298C* polymorphisms affect MTHFR activity. Enzyme activity is substantially lowered in individuals with the *677TT* genotype (homozygous variant) and less so in those with the *1298CC* genotype (homozygous variant).

cancer and adenoma in different populations, and discuss the implications of the *MTHFR* polymorphisms with respect to nutritional status in colorectal carcinogenesis. Statistical assessment in this review was carried out by using STATA statistical software version 8.0 (STATA Corporation, College Station, TX).

MTHFR polymorphisms and colorectal cancer risk

C677T polymorphism

A total of 16 studies have addressed the association between *MTHFR C677T* polymorphism and the risk of colorectal cancer in 10 countries (Table 1).[18–33] The first study was a case-control study nested in a cohort of male health professionals in the US.[18] The same research group reported another nested case-control study in the Physicians' Health Study.[19] The largest study is a case-control study nested in combined cohorts in Norway.[27] There is also a Chinese study nested in a cohort, which comprised participants in a colorectal cancer screening program.[29] Of the remaining 12 studies, five are completely or nearly population-based case-control studies,[20–22,24,33] and hospital patients were recruited as controls in two studies.[31,32] In the other five studies,[23,25,26,28,30] the source of controls and their selection were not specifically documented, although they were described as asymptomatic subjects, healthy blood donors, or healthy controls.

Prevalence of the variant allele (*677T*) varies with population and ethnicity. The *677T* allele accounted for 45% of the population in Mexico and Italy, 40% in Asian people, 30–35% in Caucasians, and 10% in Africans. The genotype distribution in each study does not measurably deviate from the Hardy–Weinberg equilibrium. In a study carried out in Hawaii[21] and in a study by Keku *et al.*,[22] the subjects had different racial backgrounds with different allele frequencies. However, the association between *C677T* polymorphism and colorectal cancer risk did not measurably differ in accordance with ethnicity in these studies, and thus the combined results with adjustment for ethnicity were used in the present analysis for ease of presentation.

Table 1. Summary of studies on *MTHFR C677T* and colorectal cancer

Study (year)	Country	No. of cases/ controls	Odds ratio (95% confidence interval)*		Frequency of *677T* allele†	Adjusted factors other than sex and age
			677CT	*677TT*		
Chen *et al.* (1996)[18]	US	144/627	1.02 (0.69–1.49)‡	0.57 (0.30–1.06)	0.344	Alcohol, folate, and others
Ma *et al.* (1997)[19]	US	202/326	0.98 (0.67–1.45)	0.45 (0.24–0.86)	0.353	Alcohol, BMI, PA, and others
Slattery *et al.* (1999)[20]	US	1467/1821	1.0 (0.9–1.2)	0.9 (0.7–1.1)	0.330	Alcohol, BMI, PA, and others
Le Marchand *et al.* (2002)[21]	US (Hawaii)	548/656	0.8 (0.6–1.1)	0.7 (0.5–1.0)	0.383	Race, BMI, PA, and others
Keku *et al.* (2002)[22]	US	552/868	1.1 (0.9–1.4)	0.8 (0.5–1.4)	0.228	Race
Delgado-Enciso *et al.* (2001)[23]	Mexico	74/110	1.83 (0.88–3.80)‡	1.61 (0.67–3.83)‡	0.455	None
Sachse *et al.* (2002)[24]	UK	490/592	0.83 (0.65–1.07)‡	1.23 (0.80–1.89)‡	0.313	None
Plaschke *et al.* (2003)[25]	Germany	287/346	1.28 (0.90–1.84)	1.13 (0.63–2.01)	0.340	None
Toffoli *et al.* (2003)[26]	Italy	276/279	0.92 (0.63–1.35)‡	0.72 (0.35–1.49)	0.452	None
Ulvik *et al.* (2004)[27]	Norway	2159/2190	1.01 (0.89–1.15)	0.73 (0.58–0.92)	0.299	Place
Shannon *et al.* (2001)[28]	Australia	501/1207	0.75 (0.60–0.94)‡	1.03 (0.72–1.47)‡	0.326	None
Jiang *et al.* (2004)[29]	China	125/340	1.07 (0.69–1.68)	0.62 (0.33–1.19)	0.397	None
Park *et al.* (1999)[30]	Korea	200/460	0.94 (0.65–1.36)‡	0.81 (0.48–1.38)‡	0.428	None
Kim *et al.* (2004)[31]	Korea	243/225	1.08 (0.72–1.60)	0.90 (0.49–1.64)	0.389	Alcohol, BMI, PA, and others
Matsuo *et al.* (2002)[32]	Japan	142/241	1.30 (0.62–2.10)	1.21 (0.62–2.34)	0.407	None
Yin *et al.* (2004)[33]	Japan	685/778	0.89 (0.71–1.12)	0.64 (0.47–0.89)	0.407	Alcohol and place
Combined estimate§			0.97 (0.90–1.04)	0.82 (0.72–0.93)		
Heterogeneity			$P = 0.32$	$P = 0.16$		

BMI, body mass index; PA, physical activity. *Referent is the *677CC* genotype. Chen *et al.* (1996)[18] and Toffoli *et al.* (2003)[26] used *677CC* and *677CT* combined as referent for *677TT*. †Frequency among controls. Le Marchand *et al.* (2002)[21]: Japanese (0.423), whites (0.377), and Hawaiian (0.216); Keku *et al.* (2002)[22]: white Americans (0.301) and black Americans (0.108). ‡Crude odds ratio without adjustment, even for age and sex. §Based on the random effect model.

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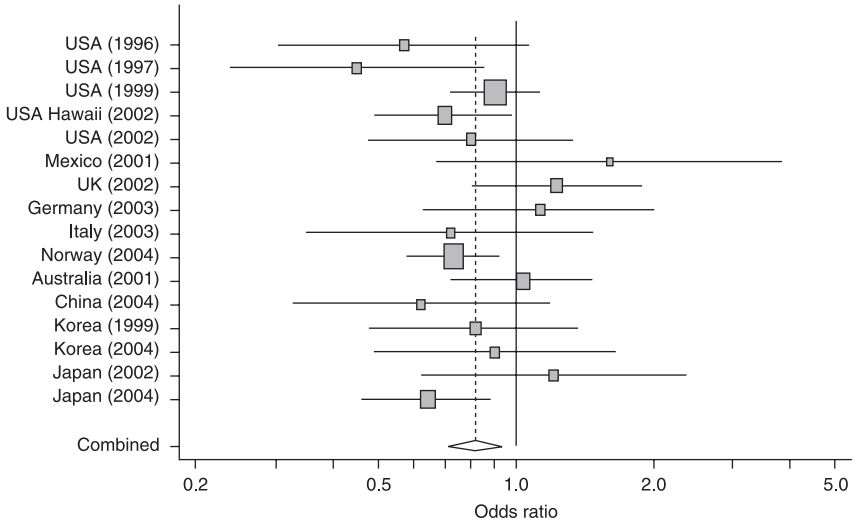

Fig. 2. Meta-analysis of 16 studies on the *MTHFR 677TT* genotype and colorectal cancer. The center of a box and the horizontal line indicate the odds ratio and the 95% confidence interval in each study, with the areas of the boxes representing the weight of each study. The summary odds ratio (random effect model) is represented by the middle of a diamond whose width indicates the 95% confidence interval. The summary odds ratio is also shown by the dotted vertical line.

In some of the studies, odds ratios were not adjusted for sex and age, and estimation of the combined odds ratio and 95% confidence interval (CI) relied upon crude odds ratios and their standard errors. The crude odds ratio would not differ much from that adjusted for sex, age, and lifestyle factors because the genotype of *C677T* is unlikely to vary according to these variables. The lack of adjustment for ethnicity may result in a biased estimate of odds ratios, however. In fact, adjusted odds ratios were not much different from crude odds ratios.

Whereas the first two studies demonstrated a large decrease in the risk of colorectal cancer associated with the *677TT* genotype, subsequent studies did not necessarily replicate the first observation (Table 1). Although individual results are seemingly variable (Fig. 2), the between-study heterogeneity is not statistically significant ($P = 0.16$). The combined odds ratio for the *677TT* versus *677CC* genotype is estimated to be 0.82 (95% CI 0.72–0.93). Overall, there is no decrease in the risk of colorectal cancer among individuals with the *677CT* genotype. Decreased risk associated with *677TT* was not observed in case-control studies in Mexico,[23] the United Kingdom,[24] Germany,[25] Australia,[28] or Japan.[32] Inconsistent findings in small studies can be ascribed to random variation, but such findings in large studies are difficult to interpret. Particularly notable is the lack of association reported in two large case-control studies in the United Kingdom[24] and Australia.[28]

Four studies have examined association with the *C677T* polymorphism by subsite of the colorectum. Two studies suggested a more marked decrease in the risk of proximal colon cancer associated with the *677TT* genotype,[20,26] but the other two found no difference in the frequency of *677TT* genotype between proximal and distal colon cancer.[28,33]

A1298C polymorphism

Few studies have examined the relationship between *MTHFR A1298C* polymorphism and colorectal cancer (Table 2).[21,22,25,29,32–35] A study reported by Chen *et al*.[34] was

Table 2. Summary of studies on *MTHFR A1298C* polymorphism and colorectal cancer

Study (year)	Country	No. of cases/ controls	Odds ratio (95% confidence interval)*		Frequency of *1298C* allele†	Adjusted factors other than sex and age
			1298AC	*1298CC*		
Chen *et al*. (2002)[34]	US	210/344	0.94 (0.64–1.39)	0.73 (0.37–1.43)	0.324	Multivitamin use and others
Le Marchand *et al*. (2002)[21]	US (Hawaii)	539/653	0.9 (0.7–1.1)	0.8 (0.5–1.4)	0.238	Racial background, BMI, PA, and others
Keku *et al*. (2002)[22]	US (white)	309/541	0.9 (0.6–1.1)	0.5 (0.3–0.8)	0.344	None
	US (black)	243/329	1.1 (0.8–1.6)	0.8 (0.3–2.1)	0.190	None
Curtin *et al*. (2004)[35]	US (men)	892/1039	1.0 (0.8–1.2)	1.0 (0.7–1.4)	0.307	BMI, PA, and others
	US (women)	703/925	1.0 (0.8–1.2)	0.6 (0.5–0.9)	0.333	BMI, PA, and others
Plaschke *et al*. (2003)[25]	Germany	287/346	1.08 (0.76–1.55)	1.42 (0.79–2.56)	0.337	None
Jiang *et al*. (2004)[29]	China	125/336	0.71 (0.44–1.14)	0.39 (0.05–3.34)	0.171	None
Matsuo *et al*. (2002)[32]	Japan	142/241	1.06 (0.76–1.67)	0.56 (0.15–2.13)	0.193	None
Yin *et al*. (2004)[33]	Japan	685/778	1.07 (0.85–1.34)	1.71 (0.93–3.14)	0.181	Alcohol and place
Combined estimate‡			0.98 (0.90–1.07)	0.83 (0.63–1.09)		
Heterogeneity			$P = 0.90$	$P = 0.02$		

BMI, body mass index; PA, physical activity. *Referent is the *1298CC* genotype. †Frequency among controls. Le Marchand *et al*. (2002)[21]: Japanese (0.210), whites (0.307), and Hawaiian (0.230). ‡Based on the random effect model.

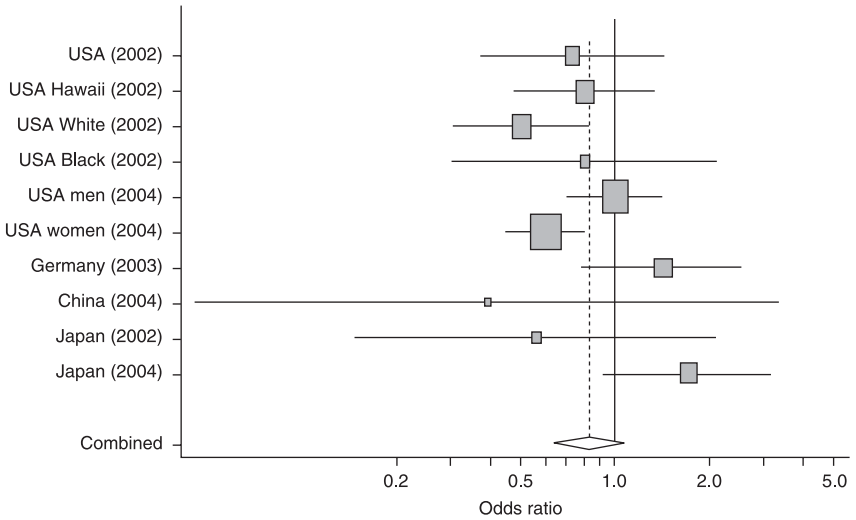

Fig. 3. Meta-analysis of eight studies on *MTHFR 1298CC* genotype and colorectal cancer. The center of a box and the horizontal line indicate the odds ratio and the 95% confidence interval in each study, with the areas of the boxes representing the weight of each study. The summary odds ratio (random effect model) is represented by the middle of a diamond whose width indicates the 95% confidence interval. The summary odds ratio is also shown by the dotted vertical line.

a case-control study in the Physicians' Health Study, and that by Curtin *et al*.[35] is the multicenter case-control study in the US that reported on MTHFR *C677T* as previously discussed.[20] All of the other studies reported the association with *A1298C* as well as with *C677T* polymorphism. The frequency of the variant *1298C* allele is much lower in Japanese and Chinese people (< 20%) than in Caucasians or populations consisting of predominantly Caucasians (30–35%). Again, none of the studies indicates a deviation of the genotype distribution from the Hardy–Weinberg equilibrium.

Although the heterozygous genotype (*1298AC*) is consistently unrelated to the risk of colorectal cancer, the association with the homozygous variant (*1298CC*) is quite variable (Fig. 3). The combined odds ratio for *1298CC* versus *1296AA* is 0.83 (95% CI 0.63–1.09). The overall decrease in the risk associated with *1298CC* is largely influenced by results from the subgroup analysis in two studies, that is, a decreased risk observed in white Americans in one study[22] and in women in another study.[35]

Combined genotype of *C677T* and *A1298C*

The *MTHFR C677T* and *A1298C* polymorphisms are in linkage disequilibrium, and combinations of *677CT* and *1298CC*, *677TT* and *1298AC*, and *677TT* and *1298CC* are null or almost null.[22,25,29,33–35] Thus an independent effect of *1298CC* (or *677TT*) is only examined in individuals with the *677CC* (or *1298AA*) genotype. Overall, the eight observations in six studies indicate that having one variant allele of either *677T* or *1298C* does not affect the risk of colorectal cancer (Table 3). The MTHFR activity of individuals with combined heterozygosity (*677CT* and *1298AC*) may be lowered to the extent that is observed among those with the *677TT* genotype,[17] but the combined heterozygosity does not seem to be functionally relevant to colorectal cancer risk.

As mentioned earlier, the *1298CC* genotype was associated with a decreased risk of colorectal cancer in subgroups of the study subjects,[22,35] and the relative importance of *677TT* and *1298CC* genotypes may be of some interest. Overall, a decreased risk of colorectal cancer is greater for *677TT* than for *1298CC*, although the 95% CI of the two odds ratios overlap to a large extent. Furthermore, the results for the combination of *677TT* and *1298AA* are more consistent than those for the composite genotype of *677CC* and *1298CC*. The two studies reporting inconsistent results within each study suggest that *1298CC* rather than *677TT* may be more important in certain situations.[22,35] These results from the subgroup

Table 3. Colorectal cancer risk according to combined genotypes of *MTHFR C677T* and *A1298C* polymorphisms

Study	Country	No. of cases/ controls	Adjusted odds ration (95% confidence interval)*				
			677CC + 1298AC	*677CC + 1298CC*	*677CT + 1298AA*	*677CT + 1298AC*	*677TT + 1298AA*
Chen *et al*. (2002)[34]	US	201/325	0.98 (0.54–1.78)	0.78 (0.36–1.72)	1.16 (0.62–2.18)	1.04 (0.57–1.92)	0.52 (0.25–1.10)
Keku *et al*. (2002)[22]	US (white)	306/536	0.9 (0.6–1.4)	0.5 (0.2–0.8)	1.1 (0.7–1.7)	0.8 (0.5–1.3)	0.8 (0.4–1.4)
	US (black)	243/327	1.1 (0.8–1.7)	0.8 (0.3–2.1)	1.0 (0.6–1.7)	0.9 (0.4–2.0)	0.8 (0.2–3.2)
Curtin *et al*. (2001)[35]	US (men)	892/1039	0.9 (0.6–1.2)	1.0 (0.7–1.4)	1.0 (0.7–1.3)	1.0 (0.7–1.4)	0.7 (0.5–1.0)
	US (women)	705/925	1.0 (0.7–1.4)	0.6 (0.4–0.9)	0.9 (0.6–1.3)	0.9 (0.6–1.2)	0.8 (0.5–1.2)
Plaschke *et al*. (2003)[25]	Germany	287/346	1.12 (0.64–1.96)	1.41 (0.74–2.71)	1.29 (0.74–2.25)	1.41 (0.81–2.45)	1.18 (0.62–2.24)
Jiang *et al*. (2004)[29]	China	124/327	0.73 (0.37–1.45)	0.54 (0.06–5.06)	1.06 (0.62–1.82)	0.67 (0.32–1.40)	0.57 (0.28–1.17)
Yin *et al*. (2004)[33]	Japan	685/778	0.93 (0.65–1.32)	1.53 (0.80–2.95)	0.89 (0.66–1.22)	0.90 (0.62–1.31)	0.64 (0.44–0.94)
Combined estimate†			0.96 (0.83–1.12)	0.87 (0.65–1.16)	1.00 (0.86–1.16)	0.94 (0.80–1.11)	0.72 (0.60–0.86)
Heterogeneity			$P = 0.97$	$P = 0.12$	$P = 0.96$	$P = 0.82$	$P = 0.76$

*Referent is the *677CC* and *1298AA* genotypes. Adjusted factors are the same as described in Table 2. †Based on the random effect model.

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analysis need further confirmation, however. No plausible explanation has been given for the inconsistency within a study.[22,35]

Effect modification of folate

A decreased risk of colorectal cancer associated with *677TT* was more evident in health professionals with high folate intake (≥ 461 μg/day) and in physicians with high folate levels in plasma (≥ 3.0 ng/mL) in the US.[18,19] A suggested effect modification of dietary folate intake in the same direction was observed in two of the three subsequent studies in the US.[20,21] However, a decreased risk associated with *677TT* was seen in both white Americans and African Americans with low folate intake (< 400 μg/day) in the study reported by Keku *et al*.[22] In that study, there was virtually no difference in the risk according to *C677T* polymorphism in individuals with high folate intake (≥ 400 μg/day). Curtin *et al*. examined the effect modification of folate on the risk associated with the composite genotype of *C677T* and *A1298C*, and showed a decrease in the risk associated with *677TT* (compared with *677CC*) in women with the *1298AA* genotype who had low folate intake (≤ 273 μg/day), with no difference in the risk between *677TT* and *677CC* genotypes in women with the *1298AA* genotype who had intermediate and high (> 388 μg/day) folate intake.[35]

Importantly, these apparently inconsistent findings are not incompatible with the role of *MTHFR C677T* polymorphism in colorectal carcinogenesis with respect to the size of the thymidylate pool. Low activity of MTHFR or the *677TT* genotype is probably advantageous because it ensures an adequate thymidylate pool for DNA synthesis when folate supply is sufficient, as originally proposed by Chen *et al*.[18] In the folate-depleted situation, as suggested by Keku *et al*.,[22] high activity of the enzyme or the *677CC* genotype may be disadvantageous because 5,10-methylenetetrahydrofolate is converted and the thymidylate pool is depleted. Increased risk for *677TT* versus *677CC* would be seen in the folate-depleted situation if aberrant DNA methylation is a primary mechanism, but no such increase has been observed.

Two studies have examined the interaction between *A1298C* polymorphism and folate. The decreased risk associated with *1298CC* observed in white Americans in the study by Keku *et al*.[22] seemed more marked when folate intake was low, and that observed in women in the study by Curtin *et al*.[35] was more evident when folate intake was high.

Effect modification of vitamins B_6 and B_{12}

Vitamins B_6 and B_{12} are important cofactors in folate metabolism. Vitamin B_6 is required for recycling tetrahydrofolate (a product of 5-methyltrahydrofolate after methyl-transfer to homocysteine) to 5,10-methylenetetrahydrofolate. Vitamin B_{12} is a cofactor of methionine synthase, and converts homocysteine to methionine. The limited results available regarding the effect modification of vitamin B_6 and B_{12} were generally similar to those observed for folate,[20,21,35] with the exception of the lack of interaction with B_{12} in the study in Hawaii.[21] This similarity is not likely to be derived from intercorrelation between intakes of these vitamins. The major food sources of folate are vegetables and fruits, but vitamin B_{12} is present primarily in animal foods, and vitamin B_6 is present in diverse foods of both animal and plant origin, as illustrated by food sources of these vitamins in the Japanese diet (Table 4).[36] With regard to methionine, the study of health professionals showed a more marked decrease in the risk associated with *677TT* in those with high methionine intake,[18] but the decrease was more marked in those with low methionine intake in Hawaii[21] and did not differ by methionine intake in the multicenter study in the US.[20]

Table 4. Average per capita intake of vitamins B_6 and B_{12} and folate in Japan, 2001

Food group	Amount (g/day)	Vitamin B_6 (mg/day)	Vitamin B_{12} (μg/day)	Folate (μg/day)
Cereals	464	0.10 (8.5)	0.01 (0.1)	27.8 (8.9)
Legumes	57	0.05 (4.2)	–	15.6 (5.0)
Vegetables	279	0.21 (17.8)	–	115.4 (36.8)
Fruits	132	0.11 (9.3)	–	21.5 (6.9)
Fish and shellfish	94	0.22 (18.6)	5.64 (73.4)	11.6 (3.7)
Meat	76	0.17 (14.4)	0.82 (10.7)	11.5 (3.7)
Eggs	37	0.03 (2.5)	0.34 (4.4)	13.6 (4.3)
Dairy foods	170	0.04 (3.4)	0.44 (5.7)	6.4 (2.0)
Tea (fluid)	301	0.03 (2.5)	–	37.7 (12.0)
Others	432	0.22 (18.6)	0.43 (5.6)	52.2 (16.7)
Total	2042	1.18	7.68	313.3

Values in parentheses are percentage nutrient intake from individual food groups. Content of each nutrient per unit amount of each food group can be obtained by dividing nutrient intake by intake of food group. Source: National Nutrition Survey in Japan, 2001.[36]

Effect modification of alcohol

Excessive alcohol consumption causes folate deficiency, as exemplified by megaloblastic anemia among chronic alcoholic abusers. Alcohol consumption leads to folate depletion by decreasing intestinal absorption and hepatic uptake, increasing renal excretion, and cleaving folate.[37] Folate deficiency may explain part of the moderate increase in the risk of colorectal cancer associated with alcohol use.[38] Studies of health professionals and physicians in the US demonstrated that risk of colorectal cancer associated with the *677TT* genotype was more markedly decreased among those with low alcohol intake.[18,19] This observation was confirmed in a case-control study in Japan,[33] but not in other studies in the US.[20,35] Three case-control studies also examined the effect modification of alcohol use on the association with *C677T* or *A1298C* in the US,[22] China,[29] and Korea,[31] but the results from these studies are not informative because alcohol consumption was extremely low[22] or was not quantified,[29] and because the *677CT* and *677TT* genotypes were collapsed into one group.[31]

Interpretation

Decreased risk of colorectal cancer associated with the *677TT* genotype has been observed in different populations with few exceptions. This decrease was observable in either high or low folate status. The thymidylate pool associated with MTHFR activity is a probable mechanism underlying the decreased risk for the *677TT* versus *677CC* genotype. The effect modification of nutritional factors such as folate and alcohol remains possible, even when there is no overall

inverse association between *677TT* genotype and colorectal cancer. Inconsistently observed decreases in the risk associated with the *A1298C* polymorphism need to be corroborated in other large studies. The effect modification of nutritional factors other than alcohol has been examined exclusively in the US, and further studies in different countries will elucidate the role of folate metabolism in colorectal carcinogenesis from a global perspective.

MTHFR polymorphisms and colorectal adenoma risk

C677T polymorphism

The association between the *MTHFR C677T* polymorphism and colorectal adenoma has been examined in eight studies in the US,[39–42] Mexico,[23] Japan,[43,44] and Norway.[45] A study of 257 adenoma cases and 713 controls in a cohort of female nurses in the US was the first to investigate the relationship between *MTHFR C677T* polymorphism and colorectal adenoma, and found no association.[39] In this study, the controls were women undergoing sigmoidoscopy who had not been diagnosed with colorectal adenoma. The lack of association between *C677T* polymorphism and colorectal adenoma was further noted in three studies of 527 cases and 645 controls, of 471 cases and 510 controls, and of 379 cases and 726 controls in the US[40–42] and in two studies of 205 cases and 220 controls and of 452 cases and 1050 controls in Japan.[43,44] All of these studies were on the basis of colonoscopy or sigmoidoscopy. In the above-mentioned Mexican study of colorectal cancer,[23] 31 cases of colorectal adenoma were included separately, and no clear association with C*677T* polymorphism was observed. On the basis of these seven studies, the pooled estimate of odds ratio for *677TT* versus *677CC* (or *677CC* and *677CT* combined) is 1.02 (95% CI 0.85–1.22). However, in a small study of 443 subjects undergoing colonoscopy in Norway,[45] the *677TT* genotype was associated with an increased risk of high-risk adenoma (defined as adenomas ≥ 10 mm in size or adenomas with villous components or severe dysplasia); there were 47 cases with high-risk adenoma, and the odds ratio for the *677TT* versus *677CC* genotype was 2.41 (95% CI 0.82–7.06) with adjustment for sex, age, erythrocyte folate, and others.

A1298C polymorphism

Only one study examined the association of this polymorphism with colorectal adenoma, and found no overall association.[42]

Effect modification of nutritional status

Despite the overall lack of association with *C677T* polymorphism, two studies in the US found a statistically significant increase in the risk of colorectal adenoma among those with the *677TT* genotype who had a high alcohol intake, which was defined as > 9.5 g/day in one study[41] and as > 30 g/day in the other.[42] A similar, but less evident, finding was also noted in a Japanese study.[43] Inconsistently, a statistically significant increase in risk was noted among those with the *677CC* genotype who had a high alcohol intake (> 7 g/day) in one study,[40] and among female nurses with the *677TT* genotype who had low alcohol intake in another study.[39] The latter finding was interpreted as being probably due to chance, but no clear explanation is evident for the former findings.

In the study reported by Ulrich *et al*., low intake of folate, vitamin B_{12}, vitamin B_6, and methionine tended to be associated with an increased risk of colorectal adenoma among those with the *677TT* genotype, while high intakes of these nutrients tended to be associated with lower risk among those with the *677TT* genotype.[40] Those with the *677TT* genotype had somewhat elevated risk when plasma folate was low and decreased risk when plasma folate was high in the US[41] as well as in Japan.[46] A substantial increase in the risk of high-risk adenoma was reported among those with the *677TT* genotype with low folate status in erythrocytes in Norway.[45] There was no clear interaction between folate intake and 677TT genotype in studies of nurses and health professionals in the US,[39,42] but the combination of high alcohol and low folate intake was associated with an increased risk, while the opposite combination was related to a decreased risk in the latter study.[42]

Interpretation

The reported decrease in adenoma risk associated with high folate or low alcohol intake among those with the *677TT* genotype is small and no more than suggestive of an association. In contrast with the observation regarding colorectal cancer, the *677TT* genotype was associated with increased risk of colorectal adenoma under conditions of low folate or high alcohol intake. Folate depletion results in a decrease in *de novo* synthesis of methionine as well as an insufficient pool of thymidylate, but DNA hypomethylation associated with low folate status seems to be limited to individuals with the *677TT* genotype.[47] Thus the increased risk observed for the combination of poor folate status and *677TT* genotype could be interpreted as suggesting that reduced availability of methyl groups for DNA methylation might be more relevant to adenoma formation rather than the progression from adenoma to carcinoma.

Conclusion

Decreased risk of colorectal cancer associated with the *MTHFR 677TT* genotype has fairly consistently been observed in different populations, and the effect of *MTHFR C677T* polymorphism on colorectal cancer risk may differ in accordance with folate status. Without consideration of the interaction between *MTHFR* polymorphism and nutritional factors, it would be concluded that folate metabolism is unrelated to the occurrence of colorectal adenoma. However, the results from analysis of the interaction between *MTHFR* polymorphism and folate or alcohol intake suggest that folate metabolism is involved in an important way in the occurrence of colorectal adenoma as well. Different patterns in the effect of the interaction between *MTHFR C677T* polymorphism and folate or alcohol intake on risks of colorectal cancer and adenoma suggest that DNA synthesis and methylation may be differently relevant to early and late stages of colorectal tumorigenesis. Although the underlying mechanisms still remain to be clarified, epidemiological findings regarding *MTHFR C677T* polymorphism provide strong evidence that adequate folate status confers protection from colorectal cancer.

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Epidemiological studies of functional genetic polymorphisms are very useful for understanding the role of dietary factors in carcinogenesis with respect to both biological mechanisms and prevention. Measurement of food and nutrient intake is always prone to a sizable random variation, unintentionally adding to the degree of homogeneity in terms of a factor under study, which in turn causes difficulties in detecting an effect of the factor. It goes without saying that a study should be large enough to address a specific question it is purported to answer, as recognized in this review.

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