Methylenetetrahydrofolate reductase C677T and *A1298C* polymorphisms and colorectal cancer: The Fukuoka Colorectal Cancer Study

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Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme regulating folate metabolism, which affects DNA synthesis and methylation. This study investigated the relation of *MTHFR C677T* and *A1298C* polymorphisms to colorectal cancer in a case-control study in Fukuoka, Japan. The subjects comprised 685 incident cases of histologically confirmed colorectal adenocarcinomas and 778 community controls selected randomly in the study area. The genotype was determined by the PCR-RFLP method using genomic DNA extracted from buffy coat. Alcohol use was ascertained by in-person interview. Statistical adjustment was made for gender, age class, area, and alcohol use. The *MTHFR 677TT* genotype was associated with a statistically significant decrease in the risk with an adjusted odds ratio of 0.69 (95% confidence interval 0.51–0.93) compared with the *677CC* and *677CT* combined, and the decrease was most evident in individuals with no alcohol consumption. While the *A1298C* polymorphism showed no measurable association with the overall risk of colorectal cancer, the *1298CC* genotype was associated with a statistically significant increase in the risk when alcohol consumption was high, and was also associated with an approximately 2-fold increase in the risk of each of proximal and distal colon cancer. The findings add to evidence that individuals with the *MTHFR 677TT* genotype have a decreased risk of colorectal cancer in the absence of folate depletion, suggesting a protective role of folate by ensuring a sufficient thymidylate pool for DNA synthesis. Because very few individuals had the *1298CC* genotype, the findings regarding the *A1298C* polymorphism need careful interpretation and confirmation in larger studies. (Cancer Science 2004; 95: 908–913)

Much attention has recently been drawn to the role of folate metabolism in colorectal carcinogenesis.[1, 2] Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme regulating folate metabolism. It irreversibly converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is the major form of folate in blood.[2] The substrate of MTHFR, 5,10-methylenetetrahydrofolate, is required for conversion of deoxyuridylate to thymidylate. Depletion of 5,10-methylenetetrahydrofolate results in uracil misincorporation into DNA, and removal of this abnormal base may lead to single and double strand breaks.[3, 4] Furthermore, insufficient thymidylate can increase DNA misrepair, resulting in overall DNA damage in the cell.[5] On the other hand, 5-methyltetrahydrofolate provides the methyl group for methylation of homocysteine to methionine. Imbalanced DNA methylation, i.e., global genomic hypomethylation and methylation of usually unmethylated CpG sites, has been implicated in colorectal carcinogenesis.[6–8]

Two common functional polymorphisms are known in the *MTHFR* gene; one is the *C677T* polymorphism in exon 4, resulting in an alanine-to-valine substitution at codon 222,[9] and the other is the *A1298C* in exon 7, resulting in a substitution of glutamate with alanine at codon 429.[10] Individuals who are homozygous for the variant allele of the *MTHFR C677T* polymorphism have been shown to have no less than 30% of normal enzyme activity, and heterozygotes (*CT*) have been shown to have 65% of normal enzyme activity.[9] As regards the *MTHFR A1298C* polymorphism, individuals with the *1298CC* genotype have been shown to have 60% of the enzyme activity of those with the *AA* genotype.[10]

Two early studies in the United States showed a decreased risk of colorectal cancer associated with *MTHFR 677TT* genotype, especially in individuals with high folate intake and with low alcohol intake.[11, 12] A consistent, but less evident, association was reported in two subsequent case-control studies in the United States.[13, 14] However, other case-control studies have failed to substantiate a protective association with the *677TT* genotype in various countries, including the United States.[15–21] Few studies have addressed the association between the *MTHFR A1298C* polymorphism and colorectal cancer.[14–16, 22] Of these, only one study showed a decreased risk of colorectal cancer associated with the *1298CC* genotype.[15]

Here, we report the relation of the *MTHFR C677T* and *A1298C* polymorphisms to colorectal cancer in a case-control study. We also examined the interaction of these polymorphisms and alcohol consumption on the risk of colorectal cancer, because alcohol is known to exert adverse effects on folate metabolism.[23] Further, the relation to these polymorphisms was examined by subsite of the colorectum, because previous studies suggested a stronger association of *C677T* with proximal colon cancer.[13, 18]

Materials and Methods

A case-control study was designed to examine the relation of lifestyle factors and genetic susceptibility to the risk of colorectal cancer. Cases were recruited from eight large hospitals in the study area (Fukuoka City and three adjacent areas), and controls were randomly selected in the community by frequency-matching to the distribution of incident cases with respect to sex and 10-year age class. The study protocol was approved by

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the ethical committees of the Faculty of Medical Sciences, Kyushu University and of all but two of the participating hospitals. Those two hospitals had no ethical committee at the time of the survey, and the survey was conducted at those hospitals with permission from the director of each hospital. This procedure conformed to the guidelines of the ethical committee of Kyushu University.

Subjects. Cases comprised a consecutive series of patients with histologically confirmed incident colorectal adenocarcinomas, who were admitted to two university hospitals or six affiliated hospitals for surgical treatment during the period from October 2000 to December 2003. Other eligibility criteria included the following characteristics: age of 20–74 years at the time of diagnosis, residence in the study area, no prior history of partial or total removal of the colorectum, familial adenomatous polyposis or inflammatory bowel disease, and mental competence to give informed consent and to complete the interview. Research nurses visited each hospital weekly, and determined the eligibility of cases by referring to admission logs and medical records. Research nurses contacted each eligible patient with permission from an attending doctor, and interviewed the patient after obtaining written informed consent.

Of 1053 eligible cases, a total of 840 cases (80%) participated in the interview, and 685 out of them gave informed consent to genotyping. Reasons for nonparticipation were patient refusal (n=115), refusal of patient's physician (n=46), and failure to make contact (n=52).

Eligibility criteria for controls were the same as described for cases except for two items, i.e., having no diagnosis of colorectal cancer and age of 20–74 years at the time of selection. A total of 1500 persons were selected as control candidates by two-stage random sampling. Numbers of control candidates by sex and 10-year age class were determined in accordance with sex- and age-specific numbers of incident cases of colorectal cancer in the Osaka Cancer Registry during the period 1988 to 1992.[24] The first step was a random selection of 15 small areas out of 178 in total. The small areas roughly corresponded to primary-school zones, merged with sparse-population zones. Approximately 100 persons were randomly selected in each small area using the municipal resident registry, with allowance for proportions of residents for each small area by sex and 10-year age class.

A letter of invitation was sent to each candidate, and a telephone call was made if the candidate was listed in the telephone directory. At most three additional letters of invitation were mailed to nonrespondents. A total of 833 persons participated in the survey, and 778 gave informed consent to genotyping. Reasons for exclusion and nonparticipation were death (n=7), migration from the study area (n=22), undelivered mail (n=44), mental incompetence (n=19), history of partial or total removal of the colorectum (n=21), diagnosis of colorectal cancer after the survey (n=5), no response (n=158), and refusal (n=391). After exclusion of the first six categories of outcomes (n=118), the net participation rate was calculated as 60% (833/1382).

Neither ethnicity nor nationality was specifically elicited in the survey, but almost all of the eligible cases and control candidates were considered to be Japanese in ethnicity, based on their names.

Interview. Research nurses interviewed cases and controls in person regarding physical activity, smoking, alcohol use, parental history of colorectal cancer, past history of selected diseases, and bowel habit by using a uniform questionnaire. Most of the questions were closed-ended, though some of the quantitative questions were open-ended. Average annual alcohol consumption at the time of 5 years prior to the interview was ascertained. Individuals reported the average number of days per week that alcohol was consumed and the average amount of alcohol per day of drinking alcohol. The amount of alcohol was expressed in the conventional unit; one *go* (180 ml) of *sake*, one large bottle (633 ml) of beer, and half a *go* (90 ml) of *shochu* were each expressed as one unit; and one drink (30 ml) of whisky or brandy and one glass (100 ml) of wine were each converted to half a unit. Reproducibility of the questionnaire was tested on 29 control subjects (14 men and 15 women) with an interval of approximately 1 year, and the reported alcohol intake was highly reproducible (Spearman's r=0.82). The cases were interviewed before or after surgery in the hospital wards, and the interview of controls was done at community halls, clinics, work place, home, or Kyushu University. A sample of venous blood (5 ml) was taken after the interview.

Genotyping. DNA was extracted from the buffy coat by using a commercial kit (Qiagen GmbH, Hilden, Germany). Genotyping was done by one of the authors (GY) using the PCR-RFLP method. The PCR was performed in a reaction mixture of 10 μl containing 0.5 units of *Taq* and 1 μl of template DNA with a concentration of approximately 50–150 ng/μl. The *MTHFR C677T* genotype was determined, as described by Fross *et al*.,[9] by using primers 5′-TGAAG GAGAA GGTGT CTGCG GGA-3′ and 5′-AGGAC GGTGC GGTGA GAGTG-3′. After the initial denaturation at 94°C for 5 min, 30 cycles of PCR were performed for 30 s at 94°C, for 30 s at 62°C, and for 30 s at 72°C, with a final extension at 72°C for 5 min. The PCR product was digested with 12 units of *Hin*fI for 3 h at 37°C in a mixture of 20 μl, which cleaves the 198-bp PCR product into two fragments of 175 and 23 bp when the *C677T* mutation exists. The digested PCR products were separated by electrophoresis on a 3% agarose gel (NuSieve GTG), and visualized with ethidium bromide.

The *MTHFR A1298C* genotype was determined by the method described elsewhere.[10, 14] Primers were 5′-CTTTG GGGAG CTGAA GGACT ACTAC-3′(sense) and 5′-CACTT TGTGA CCATT CCGGT TTG-3′ (antisense). The PCR conditions consisted of an initial denaturation at 94°C for 5 min, 35 cycles of 94°C for 30 s, 62°C for 30 s, and 72°C for 30 s, and a final extension at 72°C for 10 min. The PCR product of 163 bp was digested with 10 units of *Mbo*II in a reaction mixture of 20 μl for 3 h at 37°C. The digestion results in fragments of 56, 31, 30, 28, and 18 bp for the *1298A* allele, and fragments of 84, 31, 30, and 18 bp for the *1298C* allele. Electrophoresis was done on 4% agarose gel (MetaPhor FMC), and the genotype was discernible by detection of the 84- and 56-bp fragments.

Statistical analysis. The association of *MTHFR* genotypes with the risk of colorectal cancer was examined by means of multiple logistic regression analysis, including indicator variables for gender, 5-year age class (the lowest class of <40 years), resident area (Fukuoka City or suburban area), and alcohol intake (0, 0.1–0.9, 1.0–1.9, or ≥2.0 units per day) as covariates. Adjusted odds ratio (OR) and 95% confidence interval (CI) were obtained from the logistic regression coefficient and its standard error for the corresponding indicator variable. In the analysis of interaction between genotype and alcohol use, genotypes *677CC* and *677CT* and genotypes *1298AA* and *1298AC* were combined, respectively, and alcohol consumption was categorized into three levels of 0, 0.1–0.9, and ≥1.0 units/day because the number of individuals in the highest alcohol category (≥2.0 units per day) was small. Statistical significance for the interaction was tested by using the likelihood ratio test comparing the logistic models with and without combined terms for the genotypes and alcohol categories. The criterion of statistical significance was a two-sided P value of less than 0.05 or a 95% CI that did not include unity. All statistical analyses were done using the SAS version 8.2 (SAS Institute, Inc., Cary, NC).

Results

Numbers of men in the 685 cases and 778 controls were 426 (62%) and 490 (63%), respectively. Mean age of the cases was 60 years (range 27–74), and that of the controls was 59 years (range 22–75). As for residence, 420 cases (61%) and 501 controls (64%) were residents in Fukuoka City. Cases of cancer of the proximal colon, distal colon, and rectum numbered 150 (22%), 232 (34%), and 290 (42%), respectively; the remaining 13 cases (2%) had cancer at multiple sites.

As regards *MTHFR C677T*, frequencies of the *CC, CT*, and *TT* genotypes among controls were 36%, 47%, and 17%, respectively. The distribution of *C677T* genotype in the controls was in agreement with the Hardy-Weinberg equilibrium (P=0.82). The *677TT* genotype was less frequent in cases than in controls, and the adjusted OR of colorectal cancer for the *677TT* genotype compared with the *677CC* genotype was statistically significantly lower than unity (Table 1). Adjusted OR for *677TT* versus *677CC* and *CT* combined was 0.69 (95% CI 0.51–0.93). On the other hand, there was no material difference in the distribution of *MTHFR A1298C* genotypes between cases and controls (Table 1). Distributions of the *A1298C* genotypes in cases and controls were each compatible with the Hardy-Weinberg equilibrium (P=0.995 in cases and P=0.29 in controls). The adjusted OR for the *1298CC* versus *1298AA* genotype was slightly greater than unity, but the increase was not statistically significant. Even the comparison for *1298CC* versus *1298AA* and *1298AC* combined did not result in a statistically significant increase (adjusted OR 1.67, 95% CI 0.91–3.06).

Men and women showed similar associations with both *C677T* and *A1298C* polymorphisms. In men, adjusted ORs (and 95% CIs) for *677CC, 677CT*, and *677TT* were 1.00 (referent), 0.95 (0.71–1.26), and 0.67 (0.45–1.01), respectively. The corresponding values for women were 1.00 (referent), 0.80 (95% CI 0.55–1.17), and 0.60 (95% CI 0.34–1.05), respectively. Adjusted ORs (and 95% CIs) for *1298AA, 1298AC*, and *1298CC* were 1.00 (referent), 1.12 (0.84–1.48), and 1.38 (0.60–3.16), respectively, in men and 1.00 (referent), 0.97 (0.66–1.41), and 1.96 (0.79–4.87), respectively, in women.

Table 2 shows the distribution of combined genotypes with respect to *MTHFR C677T* and *A1298C*. The two polymorphisms were at linkage disequilibrium. No individual with the *677TT* genotype had the *1298C* allele, and only one of those having the *677CT* genotype was a variant homozygote of *1298CC*. Nonetheless, the OR for the *677TT* genotype was significantly decreased compared with the *677CC* genotype in the

Table 1. Adjusted odds ratios (OR) and 95% confidence intervals (CI) of colorectal cancer according to *MTHFR C677T* and *A1298C* polymorphisms

MTHFR genotype	Number (%)		Adjusted OR[1] (95% CI)
	Cases	Controls	
C677T			
CC	270 (39.4)	278 (35.7)	1.00 (referent)
CT	330 (48.2)	367 (47.2)	0.89 (0.71–1.12)
TT	85 (12.4)	133 (17.1)	0.64 (0.47–0.89)
A1298C			
AA	438 (64.0)	515 (66.2)	1.00 (referent)
AC	220 (32.1)	244 (31.4)	1.07 (0.85–1.34)
CC	27 (3.9)	19 (2.4)	1.71 (0.93–3.14)

1) Adjusted for gender, 5-year age class, area, and alcohol use.

Table 2. Adjusted odds ratios (OR) and 95% confidence intervals (CI) of colorectal cancer according to *MTHFR C677T* and *A1298C* genotypes in combination

MTHFR C677T		*MTHFR A1298C*		
		AA	*AC*	*CC*
CC	No.[1]	123/126	120/134	27/18
	OR (95% CI)[2]	1.00 (referent)	0.93 (0.65–1.32)	1.53 (0.80–2.95)
CT	No.	230/256	100/110	0/1
	OR (95% CI)	0.89 (0.66–1.22)	0.90 (0.62–1.31)	—
TT	No.	85/133	0/0	0/0
	OR (95% CI)	0.64 (0.44–0.94)	—	—

1) Numbers of cases/controls.
2) Adjusted for gender, 5-year age class, area, and alcohol use.

Table 3. Adjusted odds ratios (OR) and 95% confidence intervals (CI) of colorectal cancer in relation to the *MTHFR C677T* and *A1298C* genotype and alcohol use in combination

MTHFR genotype		Alcohol intake (unit/day)[1]		
		0	<1	1+
C677T				
CC+CT	No.[2]	241/254	124/158	235/233
	OR (95% CI)[3]	1.00 (referent)	0.91 (0.67–1.23)	1.15 (0.85–1.54)
TT	No.	31/57	20/31	34/45
	OR (95% CI)	0.58 (0.36–0.93)	0.73 (0.40–1.33)	0.89 (0.53–1.47)
A1298C				
AA+AC	No.	260/301	140/184	258/274
	OR (95% CI)	1.00 (referent)	0.96 (0.72–1.29)	1.18 (0.89–1.57)
CC	No.	12/10	4/5	11/4
	OR (95% CI)	1.38 (0.58–3.28)	1.03 (0.27–3.93)	3.69 (1.14–12.0)

1) One unit of alcohol intake corresponds to 1 *go* (180 ml) of *sake*, 0.5 *go* (90 ml) of *shochu*, 1 large bottle (633 ml) of beer, 2 drinks (60 ml) of whiskey, and 2 glasses (200 ml) of wine.
2) Numbers of cases/controls.
3) Adjusted for gender, 5-year age class, and area.

Table 4. Adjusted odds ratios (OR) and 95% confidence intervals (CI) of colorectal cancer according to *MTHFR C677T* and *A1298C* genotypes by subsite[1)]

MTHFR genotype	Proximal colon		Distal colon		Rectum	
	No.	OR (95% CI)	No.	OR (95% CI)	No.	OR (95% CI)
C677T						
CC	59	1.00 (referent)	95	1.00 (referent)	110	1.00 (referent)
CT	75	0.95 (0.65–1.40)	105	0.78 (0.56–1.08)	144	0.97 (0.72–1.31)
TT	16	0.58 (0.32–1.05)	32	0.65 (0.41–1.03)	36	0.67 (0.43–1.04)
A1298C						
AA	96	1.00 (referent)	140	1.00 (referent)	192	1.00 (referent)
AC	47	1.05 (0.71–1.54)	81	1.25 (0.91–1.73)	90	1.02 (0.76–1.38)
CC	7	2.09 (0.84–5.22)	11	2.36 (1.08–5.16)	8	1.15 (0.49–2.70)

1) Adjusted for gender, 5-year age class, area, and alcohol use.

group of the *1298AA* genotype, while the *A1298C* polymorphism was unrelated to colorectal cancer in the group of the *677CC* genotype. Further, individuals heterozygous with respect to both *C677T* and *A1298C* polymorphisms showed no measurable decrease in the OR as compared with wild homozygotes of the two polymorphisms.

A decrease in the OR of colorectal cancer associated with the *677TT* genotype was most evident in those with no consumption of alcohol, and the decrease was less in those with higher consumption of alcohol (Table 3). The interaction between *C677T* and alcohol use on the risk of colorectal cancer was not statistically significant, however (P=0.62). Regarding the *A1298C* polymorphism, individuals with a high alcohol consumption who had the *1298CC* genotype showed a statistically significant increase in the OR as compared with those with no alcohol consumption who had the *1298AA* or *1298AC* genotype, although the interaction was not statistically significant (P=0.41). An increase in the OR for the combination of high alcohol consumption and the *1298CC* genotype was also observed in the analysis of individuals with the *677CC* genotype; adjusted OR was 3.16 (95% CI 0.94–10.6) as compared with those with no alcohol consumption who had the *1298A* allele.

The relation of the *C677T* and *A1298C* polymorphisms to proximal colon cancer, distal colon cancer, and rectal cancer is shown in Table 4. Cases with cancer at multiple sites were excluded in this analysis. A decrease in the OR associated with the *677TT* genotype was observed for each site of cancer, although none of the decreases reached statistical significance. As regards the *A1298C* polymorphism, individuals with the *1298CC* genotype showed an approximately 2-fold increase in the OR of proximal and distal colon cancer. Similar results were also obtained in the subgroup analysis limited to individuals with the *677CC* genotype; adjusted ORs of proximal colon cancer, distal colon cancer, and rectal cancer for the *1298CC* versus *1298AA* genotype were 2.18 (95% CI 0.79–6.02), 2.07 (95% CI 0.87–4.94), and 1.04 (95% CI 0.41–2.59), respectively.

Discussion

We observed a decrease in the risk of colorectal cancer associated with the *MTHFR 677TT* genotype. The finding is in agreement with observations in several studies in the United States and in Hawaii,[11–14)] but at variance with the results from other studies in the United States,[15)] Europe,[16–18)] Australia,[19)] Korea,[20)] and Mexico.[21)] In the present study, as also observed by physicians and health professionals in the United States,[11, 12)] a protective association with the *677TT* genotype was primarily confined to those with no alcohol consumption. Alcohol consumption leads to folate depletion, probably by decreasing intestinal absorption and hepatic uptake,[23)] increasing renal excretion,[23)] and cleaving folate.[25)] The thymidylate synthesis pathway, rather than the process of DNA methylation, seems to be biologically linked with the protective association with the *677TT* genotype.[11, 12)] Under a condition of sufficient folate, low activity of MTHFR leads to buildup of 5,10-methylentetrahydrofolate, which is required for conversion of uridylate to thymidylate. An adequate pool of thymidylate decreases deoxyuridylate-induced DNA damage and ensures efficient DNA synthesis and repair.[3–5)] In this regard, inconsistency in the association with *MTHFR 677TT* genotype among studies may be related to different folate levels in different populations. Folate intake seems fairly high among adults in Japan; the average intake was estimated to be 330 μg per day in the National Nutrition Survey in 2001. This level is higher than the average intake for supplement nonusers (290 μg/day) in the period before fortification with folic acid in the United States,[26)] and is near to the average intake in the mid 1980s (400 μg/day) among health professionals in the United States.[27)]

Two previous studies showed that the *MTHFR 677TT* genotype was more strongly[13)] or exclusively[18)] associated with decreased risk of proximal colon cancer. The site-specific analysis is of interest because different molecular alterations have been implicated in carcinogenesis of the proximal and distal sites of the colorectum.[28)] Genetic alterations such as *K-ras* and *p53* mutations were shown to be more frequent in the distal site, while microsatellite instability (MSI) was almost exclusively associated with proximal colon cancer.[29–32)] Interestingly, the *MTHFR 677TT* genotype was shown to be positively associated with MSI-positive colorectal cancer, but not with MSI-negative cancer.[19)] In the present study, however, a decreased risk associated with the *677TT* genotype was observed for both distal colon cancer and rectal cancer, as well as for proximal colon cancer.

Few studies have previously examined the relation between the *MTHFR A1298C* polymorphism and colorectal cancer. A study in the United States showed a statistically significant decrease in the risk of colon cancer for the *1298CC* genotype compared with the *1298AA* genotype, while showing no measurable decrease in the risk associated with the *MTHFR 677TT* genotype.[15)] Two other studies suggested a slightly decreased risk of colon or colorectal cancer associated with the *1298CC* genotype among physicians in the United States[22)] and in Hawaii,[14)] whereas the *1298CC* genotype was associated with a statistically nonsignificant increase in the risk in Germany.[16)] In the present study, individuals with the *1298CC* genotype showed a small, statistically nonsignificant increase in the overall risk of colorectal cancer and a significant increase in the risk when alcohol consumption was high. Moreover, a 2-fold increase in the risk associated with the *1298CC* genotype was noted for proximal and distal colon cancer. These findings are, however, difficult to interpret because of the small numbers of individuals with the *1298CC* genotype in the subgroup analysis. Further studies are needed to confirm the present findings with

regard to the *A1298C* polymorphism.

As reported in many different populations,[33] the *A1298C* polymorphism was at linkage disequilibrium with the *MTHFR C677T* polymorphism. Frequencies of the *677T* and *1298C* alleles were 41% and 18%, respectively, among the controls in the present study. These frequencies are similar to those reported in random samples of Japanese in Hawaii[14] and Japan,[34] but the frequencies of *677T* and *1298C* alleles in Japanese seem to differ from the frequencies in Caucasians. Frequencies of both the *677T* and *1298C* alleles are generally in the range of 30–35% in Caucasians.[11–19] The relatively lower frequency of the *1298C* allele makes it somewhat difficult to study the relation of *MTFHR A1298C* to colorectal cancer in Japanese.

Another point of interest was whether heterozygotes for both *MTHFR C677T* and *A1298C* polymorphisms had lower risk of colorectal cancer as compared with wild homozygotes of the two polymorphisms. Individuals with combined heterozygosity for *MTHFR 677CT* and *1298AC* showed reduced enzyme activity, elevated plasma homocysteine, and decreased plasma folate, similar to those with the *677TT* genotype.[10] However, there was no evidence that the combined genotypes of *677CT* and *1298AC* conferred a decreased risk of colorectal cancer in the present study.

Several methodological advantages of the present study deserve discussion. This is probably the second largest study that has ever been reported regarding the MTHFR genotype and colorectal cancer. Among the largest studies are a multicenter study including 1467 colon cancer cases and 1821 controls in the United States,[13] a study of 548 cases and 656 community controls in Hawaii,[14] and a study of 555 cases and 875 controls in North Carolina.[15] The size of study is particularly important in investigating the role of rare genotypes in the gene-environment, or gene-gene interaction. Also notable are the fairly high participation rates in both cases (80%) and controls (60%). It is generally argued that bias related to selection or confounding is unlikely to occur in studies of genotypes and disease because of the so-called Mendelian randomization,[35] but selection as regards environmental factors modifying the association with a specific genotype could distort the true association with the genotype. We used alcohol consumption five years prior to the interview. We have no data as to how valid the recalled alcohol consumption in the past was, although it was found to be highly reproducible. The lack of information as to folate intake was another weakness in the present study. Knowledge of the interaction between folate intake and the *MTHFR* polymorphisms would be useful in elucidating the role of the *MTHFR* polymorphisms in colorectal carcinogenesis.

In summary, a large case-control study in Japan showed a decreased risk of colorectal cancer associated with the *MTHFR 677TT* genotype, especially among individuals with no alcohol consumption. A decreased risk associated with the *MTHFR 677TT* genotype was observed for cancers of the proximal colon, distal colon, and rectum. The *MTHFR 1298CC* genotype was associated with an increased risk when alcohol consumption was high, and was also associated with increased risks of proximal and distal cancer. The latter findings need careful interpretation and confirmation in larger studies, because very few individuals had the *1298CC* genotype.

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