RESEARCH COMMUNICATION

Methionine Synthase Reductase Gene A66G Polymorphism is Associated with Risk of Colorectal Cancer

Keitaro Matsuo[1,*], Nobuyuki Hamajima[1], Takashi Hirai[2], Tomoyuki Kato[2], Manami Inoue[1], Toshiro Takezaki[1], Kazuo Tajima[1]

Abstract

A hospital-based case-control study was conducted to evaluate the significance of methionine and folate related polymorphisms, with 72 colon and 70 rectal cancer cases and 241 non-cancer controls. The polymorphisms examined were in the genes for methionine synthase reductase (*MTRR* A66G), methionine synthase (*MTR* A2756G) and methylenetetrahydrofolate reductase (*MTHFR* C677T and A1298C). An unconditional logistic regression model was applied for estimating the odds ratios (ORs) and 95% confidence intervals (CIs). The age-sex adjusted OR for the *MTRR GG* genotype as compared with the *AA* and *AG* genotypes was 2.77 (95% CI: 1.39-5.53, p = 0.004), whereas adjusted ORs for other polymorphisms were not statistically significant. When the ORs for environment factors (smoking, alcohol consumption, body-mass-index, and physical exercise) were calculated according to each polymorphism, no substantial difference was observed except with the *MTRR* polymorphism. The ORs for the *MTRR GG* genotype seemed to be modified by the extent of environmental exposure. In conclusion, the present study showed that the *GG* genotype of *MTRR* A66G is a risk factor for colorectal cancer in Japanese, while *MTHFR* and *MTR* polymorphisms are not. The conclusions, however, need further evaluation in terms of micronutrient status and additional confirmatory studies are required with datasets for various ethnic groups.

Key Words: Colorectal cancer - gene polymorphism - methionine synthase reductase - folate - methionine.

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Introduction

Methylation of CpG sites inhibits DNA transcription and regulates gene expression and imbalanced DNA methylation is observed consistently in colonic neoplasia (Goelz et al., 1985, Makos et al., 1992). As shown in Figure 1, methionine and folic acid metabolic pathways play important roles in DNA methylation. Experimental models have shown deficiency in these nutrients to result in alteration of DNA methylation and eventually lead to carcinogenesis (Hoffman 1984). Epidemiologic data also support the conclusion that a low folate or low methionine diet along with high alcohol consumption may increase the risk of colon cancer development (Giovannucci et al., 1995). We therefore

hypothesized that genetic differences in the relevant metabolic pathways, such as single nucleotide polymorphisms, might predispose to colorectal cancer.

In the present study, we focused on three methionine/folate metabolizing enzyme genes, methionine synthase reductase (MSR), encoded by *MTRR* (abbreviation set by concensus = 5-methyltetrahydrofolate-homocysteine methyltransferase reductase) and located at 5p15.2-p15.3, methionine synthase (MS), encoded by *MTR* and located at 1q43 (Leclerc et al., 1996), and methylene-tetrahydrofolate reductase (MTHFR), encoded by *MTHFR* and located at 1p36 (Goyette et al., 1994), which might closely involved in the DNA methylation or DNA synthesis.

MS catalyzes the cobalamine (vitamin B_{12})-dependent

[1]*Aichi Cancer Center Research Institute, Division of Epidemiology and Prevention,* [2]*Aichi Cancer Center Hospital, Department of Gastroenterological Surgery.*
Corresponding author: Keitaro Matsuo M.D., Ph. D., Aichi Cancer Center Research Institute, Division of Epidemiology and Prevention, 1-1 Kanokoden Chikusa-ku Nagoya 464-8681, Japan. Tel:+81(52)764-2988, Fax:+81(52)763-5233, e-mail: kmatsuo@aichi-cc.jp
Contact address: 203 Park Drive, G-10, Boston, MA 02215 Tel/Fax: +1-(617)247-3627, e-mail: kmatsuo@aichi-cc.jp

methylation of homocysteine to form methionine using 5-methyl tetrahydrofolate (THF) as the methyl donor and is itself activated by MSR through reductive methylation using S-adenosylmethionine (SAM) as the methyl donor (Figure 1). The MS encoding gene, *MTR*, has an A2756G polymorphism (Leclerc et al., 1996) that may modify enzyme activity (Harmon et al., 1999). Moderate associations of the polymorphism with colorectal cancer have already been reported in Caucasians (Ma et al., 1999). The MSR encoding gene, *MTRR*, also has a possibly functional polymorphism A66G (Brown et al., 2000, Hobbs et al., 2000, Wilson et al., 1999), but to the best of our knowledge, any association with colorectal cancer has yet to be evaluated.

MTHFR catalyzes the reduction of 5,10-methylene THF to 5-methyl THF (Shane 1990), the predominant circulatory form of folate and carbon donor for re-methylation of homocysteine to methionine (Figure 1). The *MTHFR* gene is reported to have two polymorphisms, involving nucleotides 677 (C677T) and 1298 (A1298C). Both affect MTHFR activity (Frosst et al., 1995, van der Put et al., 1998), and associations in Caucasians have been reported for colorectal cancers (Ma et al., 1997, Slattery et al., 1999), and adenomas (Ulrich et al., 1999) but not for hyperplastic polyps (Ulrich et al., 2000).

Since there have been no reports for Japanese, we conducted the present hospital-based case-control study at Aichi Cancer Center (ACC) to explore associations between these polymorphisms and colorectal cancer in our population.

Methods

Study Population and Sample Collection.

The case-control study was conducted as a part of series in a major project on genetic polymorphisms and cancer risk with patients at ACC (Hamajima et al., 2001c, Takezaki et al., 2001). All cases and controls were Japanese. Cases were recruited who were confirmed histologically as having colon (n=72) or rectal (n=70) cancer, excluding those with a history of other types of malignancies. Controls (n=241) were outpatients without any history of cancer who visited ACC during March to December 1999 for gastroscopy (Hamajima et al., 2001a); including 97 (40.2% out of 241) participants stated to be under medication for various diseases (not confirmed by their medical records); 23 with gastric/duodenal ulcer, another 23 for so-called gastritis, 16 with hypertension, 8 for pain including arthritis and lumbago, 7 with diabetes mellitus, 7 with hyperlipidemia, and the other
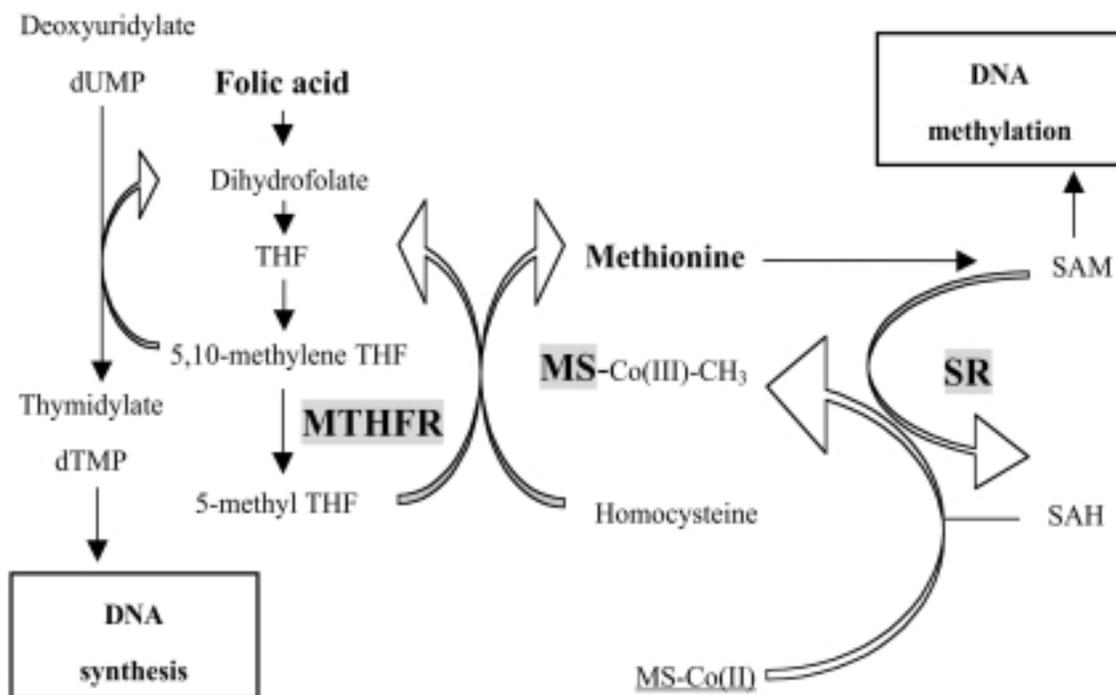
Figure 1. Overview of Methionine and Folate Metabolizing Pathways.
MS (methionine synthase) catalyzes the transfer of the methyl base from 5-methyl THF (tetrahydrofolate) to homocysteine. MSR (methionine synthase reductase) catalyzes reductive methylation of the co-factor of MS, cobalamine, using S-adenosylmethionine (SAM), and activates MS activity. MTHFR (methylenetetrahydrofolate reductase) catalyzes reduction of 5,10-methylene THF to 5-methyl THF. Reduced activity of MS leads to hypomethylation of DNA. SAH indicates S-adenosylhomocysteine. Each pathway leads to DNA methylation and synthesis.

23 with miscellaneous diseases. Subjects who provided written informed consent for participation in this study were asked to complete a self-administered questionnaire and to provide blood from a peripheral vein. This study was approved by the Ethical Committee of ACC (Approval No. 12-23 and 12-27).

Environmental Factors

Smokers were divided into three categories (never, former, and current). We defined former smokers as those who had quit smoking more than 2 years before disease onset or the questionnaire study. Habitual exercise was divided into two categories (three times or more / week and less). Alcohol drinking was also divided into three categories based on drinking frequency and quantity; low (less than once a week), moderate (once a week or more frequently with less than 2 'gou' equivalent to 50mL alcohol) or high (once a week or more frequently with 2 'gou' or more). Body mass index (BMI) was calculated as the body weight (kg) divided by squared height (m²), and classified into two categories with a value of 23.0 kg/m² as cut-off. We asked the cases to provide information about their lifestyle before the onset of disease, and the controls at the study enrollment.

Genotype Analyses of the MTRR, MTR, and MTHFR.

DNA of each subject was extracted from the buffy coat fraction with a QIAamp DNA Blood Mini Kit (Qiagen Inc., Valencia, CA).

Genotyping was performed according to previously described methods for *MTRR* A66G (Wilson et al., 1999), *MTR* A2756G (Matsuo et al., 2001), and *MTHFR* C677T (Frosst et al., 1995) and A1298C (Skibola et al., 1999) polymorphisms in detail. For the *MTRR* A66G polymorphism, DNA was amplified with the forward primer 5'-GCA AAG GCC ATC GCA GAA GAC AT-3' and the reverse primer 5'-GTG AAG ATC TGC AGA AAA TCC ATG TA-3'. PCR thermal cycling conditions were 5 min denaturation at 94°C, then 40 cycles of 94°C for 30sec, 56°C for 30sec, and 72°C for 30sec. This was followed by 7 min extension at 72°C. Amplified 66-bp PCR products were digested with *Nde* I (Boehringer Manheim; Germany) and visualized after electrophoresis on a 4% of agarose gel with ethidium bromide. The *G* allele produced a 66-bp band, while the *A* allele produced 44- and 22-bp fragments. For the *MTR* A2756G, DNA was amplified with the forward primer 5'-TGT TCC AGA CAG TTA GAT GAA AAT C-3' and the reverse primer 5'-GAT CCA AAG CCT TTT ACA CTC CTC-3'. PCR thermal cycling conditions were 5 min denaturation period at 95°C, followed by 35 cycles of 95°C for 1 min, 60°C for 1.5 min and 72°C for 1 min, and a 7 min extension at 72°C. PCR products were digested with *Hae* III (Boehringer Manheim; Germany), resulting in a 211-bp band for the A allele, and 131- and 80-bp fragment for the G allele, after 4% agarose gel electrophoresis. For the *MTHFR* 677 C to T polymorphism, DNA was amplified with the forward primer 5'-TGA AGG AGA AGG TGT CTG CGG GA-3' and the reverse primer 5'-AGG ACG GTG CGG TGA

GAG TG-3'. PCR thermal cycling conditions were 5 min denaturation at 94°C, then 40 cycles of 94°C for 30sec, 62°C for 30sec, and 72°C for 30sec. This was followed by 7min extension at 72°C. Amplified 198-bp PCR products were digested with *Hinf* I (Boehringer Manheim; Germany) and visualized under electrophoresis on a 4% of agarose gel with ethidium bromide. The C allele produced a 198-bp band, and the T allele produced 175- and 23-bp fragments. For the *MTHFR* 1298 A to C polymorphism, DNA was amplified with the forward primer 5'-CTT TGG GGA GCT GAA GGA CTA CTA C-3' and the reverse primer 5'- CAC TTT GTG ACC ATT CCG GTT TG-3'. PCR conditions were 2 min denaturation at 92°C, followed by 35 cycles of 92°C for 1 min, 60°C for 1 min, and 72°C for 30 sec, with 7 min of extension at 72°C. With 4% agarose gel visualization, *Mbo* II (New England Biolab, Inc., Beverly, MA) digestion produced 56-, 31-, 30-, 28- and 18-bp bands for the A allele, and 84-, 31-, 30- and 18-bp bands for the C allele. Heterozygotes produced bands for each allele.

Statistical Analysis

All of the statistical analyses in this study were performed using STATA version 7.0 statistical software (STATA Corporation Inc., College Station, TX). Accordance with the Hardy-Weinberg equilibrium was checked for controls with the Fisher's exact test. Categorical variables were tested with the χ2 test. All odds ratios (ORs) and 95% confidence intervals (CIs) were estimated by unconditional logistic regression. Age was adjusted as a continuous variable. Interaction in this study was defined as the ratio of the OR for the target genotype to the OR for individuals with the other genotype. Adjustment for multiple comparisons was not performed, because the analyses were conducted in an exploratory context (Bender and Lange 2001), which requires a careful interpretation of any p-values.

Results

Table 1 summarizes general characteristics of the subjects. Individuals older than 70 years of age were slightly more frequent among colon than rectal cancer cases. The percentage of current smokers was slightly higher in those with rectal cancer, but this was not statistically significant by the χ² test. High alcohol consumers predominated in male rectal cancer cases with statistical significance ($\chi^2 = 41.1$, p <0.001). The distribution of BMI and habitual exercise was not significantly different from those for controls even on subsite analysis.

Genotype distributions and sex-age adjusted ORs for each polymorphism are shown in Table 2. The Fisher's exact test for Hardy-Weinberg equilibrium was not statistically significant for all examined polymorphisms: *MTRR* (p =0.064), *MTHFR* 677 (p = 0.303), *MTHFR* 1298 (p > 0.99), and *MTR* (p = 0.276). The *MTRR* genotype frequencies for cases were 44.4%, 36.1%, and 19.4% for colon cancers, and 45.7%, 41.4%, and 12.9% for rectal cancers, the *GG* genotype thus being more frequently observed in cases. The

Table 1. Background Characteristics of Cases and Controls by Sex.

	Males			Females		
	Controls	Cases		Controls	Cases	
		Colon	Rectum		Colon	Rectum
	N=118	N=42	N=41	N=123	N=30	N=29
Number (%) of						
Age in years						
< 40	2 (1.7)	3 (7.1)	0 (0)	0 (0)	2 (6.7)	2 (6.9)
40-49	21 (17.8)	7 (16.7)	6 (14.6)	23 (18.7)	2 (6.7)	5 (17.2)
50-59	34 (28.8)	12 (28.6)	16 (39.0)	56 (45.5)	12 (40.0)	9 (31.0)
60-69	61 (51.7)	12 (28.6)	15 (36.6)	44 (35.8)	8 (26.7)	10 (34.5)
≥ 70	0 (0)	8 (19.1)	4 (9.8)	0 (0)	6 (20.0)	3 (10.3)
Years from diagnosis						
<1 year	-	5 (11.9)	10 (24.4)	-	11 (36.7)	10 (34.5)
1-2 year	-	21 (51.2)	20 (48.8)	-	10 (33.3)	14 (48.3)
≥ 2 year	-	16 (38.1)	11 (26.8)	-	9 (30.0)	5 (17.2)
Smoking status*						
Never	35 (28.8)	10 (23.8)	4 (9.8)	106 (86.2)	27 (90.0)	26 (89.7)
Former	38 (32.2)	18 (42.9)	18 (43.9)	5 (4.1)	1 (3.3)	0 (0.0)
Current	46 (39.0)	14 (33.3)	19 (46.3)	12 (9.8)	2 (6.7)	3 (10.3)
Drinking status						
< 1 time / week	35 (29.7)	9 (21.4)	6 (14.6)	101 (82.1)	26 (86.7)	26 (79.3)
1-4 times / week	30 (25.4)	11 (26.2)	4 (9.8)	15 (12.2)	1 (3.3)	4 (13.8)
< 2 gou† / drink	22	8	3	12	1	3
≥ 2 gou / drink	8	3	1	3	0	1
≥ 5 times / week	53 (44.9)	22 (52.4)	31 (75.6)	6 (4.9)	3 (10.0)	2 (6.9)
< 2 gou / drink	28	9	9	5	2	2
≥ 2 gou / drink	25	13	22	1	1	0
Unknown	0 (0)	0 (0)	0 (0)	1 (0.8)	0 (0)	0 (0)
Exercise						
< 3 times / week	84 (71.2)	34 (81.0)	29 (70.7)	89 (72.4)	23 (76.7)	25 (86.2)
≥ 3 times / week	34 (28.8)	8 (19.0)	12 (29.3)	34 (27.6)	7 (23.3)	4 (13.8)
BMI						
≤ 23 kg / m²	62 (52.5)	18 (42.9)	14 (34.2)	82 (66.7)	20 (66.7)	19 (65.5)
> 23 kg / m²	56 (47.5)	24 (57.1)	27 (65.8)	41 (33.3)	10 (33.3)	10 (34.5)

* Former smokers are defined as those who had quit smoking more than 2 years before disease onset or the questionnaire study.
† The 2-gou is equivalent to 50ml of alcohol.

OR for *MTRR GG* genotype compared with *AA* and *AG* genotypes was 2.77 (95% CI: 1.39-5.53, p = 0.004). The adjusted ORs for other polymorphisms were not statistically significant without any implied risk for colorectal cancer.

Table 3 shows the ORs for each polymorphism according to environment factors. Although there were no significant differences in ORs for *MTHFR* 677, *MTHFR* 1298 and *MTR* 2756 polymorphisms, those for *MTRR* A66G showed a certain trend with extent of environment exposure. The point estimates for smoking and alcohol drinking showed an increasing trend, while an opposite trend was observed for exercise and BMI.

To rule out any prognostic effect of these polymorphisms among cases, we estimated the OR by the interval from diagnosis (incident group: ≤ 3 years; prevalent group: > 3 years). For the colorectal cancer, the ORs for the *MTRR GG* genotype were 2.73 (.28-5.82) and 3.63 (1.31-10.0) for the incident and prevalent groups, respectively. Similar analyses for other polymorphisms and for each site also showed no differences.

Discussion

In present hospital-based case-control study, 142 cases and 241 controls were examined, so that the statistical power was more than 75% for OR=3 or 0.33 under a two-side significance level of 0.05, with a GG genotype frequency among the controls of between 5% and 15%. We found the

Table 2. Number of Cases and Controls, Sex-Age-adjusted Odds Ratios (OR) and 95% CIs for MTRR, MTHFR and MS, Polymorphisms.

Genotype	Controls	All cases		Colon cancer			Rectal cancer		
		OR	95%CI		OR	95%CI		OR	95%CI
	n=241 (%)	n=142 (%)		n=72 (%)			n=70 (%)		
MTRR A66G									
AA	112 (46.5)	64 (45.1)	1.00	Reference	32 (44.4)	1.00	Reference	32 (45.7)	1.00	Reference
AG	114 (47.3)	55 (38.7)	0.83	0.53-1.30	26 (36.1)	0.77	0.43-1.38	29 (41.4)	0.87	0.49-1.54
GG	15 (6.2)	23 (16.2)	2.53	1.23-5.23	14 (19.4)	2.91	1.26-6.74	9 (12.9)	1.99	0.79-5.01
AA/AG		1.00	Reference		1.00	Reference		1.00	Reference
GG		2.77	1.39-5.53		3.31	1.50-7.32		2.13	0.88-5.14
MTHFR C677T									
CC	81 (33.6)	39 (27.5)	1.00	Reference	23 (31.9)	1.00	Reference	16 (22.9)	1.00	Reference
CT	124 (51.5)	81 (57.0)	1.30	0.62-2.10	39 (54.2)	1.03	0.57-1.86	42 (60.0)	1.69	0.89-3.21
TT	36 (14.9)	22 (15.5)	1.21	0.62-2.34	10 (13.9)	0.92	0.39-2.17	12 (17.1)	1.64	0.70-3.8
MTHFR A1298C*									
AA	157 (65.2)	94 (66.2)	1.00	Reference	50 (69.4)	1.00	Reference	44 (62.9)	1.00	Reference
AC	75 (31.1)	44 (31.0)	1.06	0.76-1.67	19 (26.4)	0.84	0.46-1.54	25 (35.7)	1.27	0.72-2.24
CC	9 (3.7)	3 (2.1)	0.56	0.15-2.13	3 (4.2)	1.06	0.27-4.09	0 (0.0)	NE†	NE
UK	0 (0.0)	1 (0.7)			0 (0.0)			1 (1.4)		
MTR A2756G									
AA	156 (64.7)	90 (63.4)	1.00	Reference	46 (63.9)	1.00	Reference	44 (62.9)	1.00	Reference
AG	79 (32.8)	47 (33.1)	1.03	0.66-1.61	22 (30.6)	0.97	0.54-1.72	25 (35.7)	1.10	0.63-1.94
GG	6 (2.5)	5 (3.5)	1.33	0.39-4.56	4 (5.6)	1.91	0.50-7.27	1 (1.4)	0.58	0.07-5.02

* One case was excluded from analysis because DNA was not amplified by PCR.

† NE indicates 'not estimated'.

Table 3. Number of Cases and Controls, Sex-Age-adjusted Odds Ratios (ORs), 95% CIs for each Polymorphism Genotype According to Lifestyle

Genotype	*MTRR* A66G				*MTHFR* C677T				*MTHFR* A1298C				*MTR* A2756G			
	AA/AG	*GG*			*CC*	*CT/TT*			*AA*	*AC/CC*			AA	AG/*GG*		
	Ca/Co*	Ca/Co	OR	95%CI	Ca/Co	Ca/Co	OR	95%CI	Ca/Co	Ca/Co	OR	95%CI	Ca/Co	Ca/Co	OR*	195%CI
Smoking†																
Never	59/131	8/9	1.0	0.7-5.3	30/65	75/37	1.1	0.6-1.9	46/87	21/53	0.7	0.4-1.4	48/89	19/51	0.7	0.4-1.3
Former	41/32	5/2	2.6	0.5-14.7	18/21	19/22	1.0	0.4-2.5	26/32	11/11	1.6	0.4-3.6	19/30	18/13	2.4	0.9-6.2
Current	28/54	10/4	4.4	1.3-15.6	13/31	25/27	2.2	0.9-5.1	22/38	15/20	1.5	0.6-3.5	23/37	15/21	1.2	0.5-2.8
Drinking‡,§																
Low	55/126	10/9	1.0	0.8-5.1	32/67	32/69	0.9	0.5-1.7	44/84	19/52	0.7	0.4-1.4	41/90	23/46	1.1	0.6-2.1
Moderate	33/64	4/3	2.3	0.5-11.3	14/32	23/35	1.5	0.6-3.4	25/45	12/22	1.0	0.4-2.4	26/45	11/22	0.9	0.4-2.1
High	31/35	10/2	5.5	1.1-27.5	15/18	26/19	1.7	0.7-4.3	25/28	16/9	2.1	0.8-5.7	23/21	18/16	1.0	0.4-2.5
Exercise																
< 3 times/ week	89/161	22/11	3.4	1.6-7.4	45/79	66/94	1.2	0.7-2.0	71/117	39/56	1.3	0.8-2.1	71/104	40/69	0.9	0.5-1.4
≥ 3 times/ week	30/64	1/4	0.6	0.1-5.4	16/38	15/30	1.1	0.5-2.6	23/40	8/28	0.5	0.2-1.3	19/52	12/16	1.8	0.7-4.7
BMI																
≤ 23 kg/m²	58/137	13/7	4.4	1.7-11.8	32/72	39/72	1.2	0.7-2.2	40/92	31/52	1.4	0.8-2.5	49/95	22/49	0.9	0.5-1.6
> 23 kg/m²	61/89	10/8	1.7	0.6-4.8	29/45	42/52	1.2	0.6-2.3	54/65	16/32	0.7	0.3-1.4	41/61	30/36	1.3	0.7-2.5

*Ca/Co indicates cases and controls.

†Former smokers is defined as those who had quit smoking more than 2 years before disease onset or the questionnaire study.

‡Low (less than once a week), moderate (once a week or more frequently with less than 2 gou equivalent to 50mL alcohol) or high (once a week or more frequently with 2 gou or more).

§One control was excluded from analysis because information of alcohol consumption was missing.

GG genotype of the *MTRR* A66G polymorphism to triple the risk of colorectal cancer compared with the AA/AG genotypes in Japanese, while *MTHFR* 677, *MTHFR* 1298, and *MTR* 2756 polymorphisms did not alter the risk.

Elevated ORs were found for both colon and rectal cancers in the subjects with the *MTRR* 66GG genotype, suggesting the MSR pathway is related to carcinogenesis in common in both sites, the polymorphism affecting activity to a sufficient extent to change colorectal cancer susceptibility. To our knowledge, this is the first documentation of an association between this polymorphism and colorectal cancer. As shown in Figure 1, MSR activates MS by reducing the cobala(II)mine co-factor of MS to cobala(III)mine. The polymorphism is located in the FMN-binding domain (Leclerc et al., 1998), and the *G* allele is considered to decrease the enzyme activity compared with the *A* allele (Wilson et al., 1999), with resultant hypomethylation of DNA. The speculation that hypomethylation predisposes to colorectal cancer is consistent with previous observations (Goelz et al., 1985). Wilson et al suggested the polymorphism might be effective only when co-factor cobalamine is deficient (Wilson et al., 1999), but this could not be addressed in the present study. Clearly, further evaluation in terms of biology and epidemiology is desired.

Concerning *MTHFR* and colorectal diseases, five studies have been reported. Ma et al first described *MTHFR* 677T homozygotes to have a reduced risk of colorectal cancer (Ma et al., 1999). Slattery et al. also showed a weak effect of the *MTHFR* C677T polymorphism for the colorectal cancer, but suggested its effect could be modified by folate, methionine, and alcohol status (Slattery et al., 1999). Associations were also reported for colorectal adenomas (Ulrich et al., 1999), but not for hyperplastic polyps (Ulrich et al., 2000). The former 3 studies all suggested an interaction between *MTHFR* polymorphism and alcohol intake. One earlier study from Japan found no association with colorectal adenoma (Marugame et al., 2000), however, and our present data showed no link even when alcohol was considered. For the A1298C polymorphism, ours is the first report to our knowledge. Reasons for disagreement with former studies are unclear, but folate, methionine, and cobalamine status or other genetic background might blur the association.

For the *MTR* A2756G polymorphism, only one report from the United States exists (Ma et al., 1997). Subjects with the *2756GG* genotype showed a tendency for risk reduction, but its effect was weaker than that of *MTHFR* C677T polymorphism. While we observed no influence of this polymorphism, further investigation with a large number of subjects is required for firm conclusions.

Because we applied a prevalent case-control design, special attention needs to be paid to interpretation (Hamajima et al., 2001b). If the *MTRR* genotype under study had a prognostic impact, the derived ORs would be influenced. However, our result showed similar estimations warranting a conclusion that the *MTRR* genotype and the interaction terms were almost free from survival effects. In this study,

no significant Hardy-Weinberg disequilibrium was observed for the *MTRR* A66G polymorphism, indicating appropriate sampling of controls.

In conclusion, the present study showed the *GG* genotype of the *MTRR* A66G polymorphism to be a risk factor for colorectal cancer. Further evaluation of ethnicity is warranted.

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