evidence that calcium and folate are protective in the development of colorectal cancer, whereas evidence for protective associations with these factors was judged as insufficient in the report of the WCRF/AICR (1997). Randomized trials consistently observed that calcium supplementation resulted in a measurable reduction in the recurrence of colorectal adenomas, a well established precursor lesion for the colorectal cancer (Baron et al., 1999; Bonithon-Kopp et al., 2000). Further a pooled analysis of prospective data in Europe and the United States showed a deceased risk of colorectal cancer associated with calcium intake (Cho et al., 2004). Studies of a genetic polymorphism of methylenetetrahydrofolate reductase, a key enzyme in folate metabolism, have strengthened evidence for a protective role for folate in the occurrence of colorectal cancer (Matsuo et al., 2002; Sharp and Little, 2004). The pros and cons have been argued regarding red meat and colorectal cancer since the report of WCRF/AICR (1997). Hill (2001) argued against the conclusion in the WCRF/AICR report. A recent cohort study failed to find an increased risk of colon cancer associated with meat as a whole or red meat (Flood et al., 2003), whereas a meta-analysis showed a modest increase in the risk of colorectal cancer associated with red and processed meat (Norat et al., 2002). On the other hand, in an ecological study in Japan (Kono, 2004), colon cancer incidence rates were strongly correlated with meat consumption approximately 20 years earlier. Studies of metabolic polymorphisms suggested that increased risk of colorectal cancer associated with red meat consumption might be due to exposure to nitrosamines (Le Marchand et al., 2002) and heterocyclic amines (Le Marchand et al., 2001). Heme-iron intake might also have been responsible for the association between red meat and colorectal cancer as shown in another study (Lee et al., 2004).

Finally, 685 cases and 778 controls gave consent to genotyping in the present study. Studies on genetic polymorphisms of known functions not only shed light on mechanisms of environmental factors increasing or decreasing the risk of colorectal cancer, but also have the potential to provide decisive conclusions regarding inconsistent associations with environmental factors in case-control and cohort studies. Our relatively large number of colorectal cancer cases will enable us to examine lifestyle factors and genetic polymorphisms according to subsites in the colorectum. Etiological factors may vary not only between colon and rectal cancer but also between segments of the colon. Recent studies have shown different molecular alterations between proximal and distal sites of the colorectum (Breivik et al., 1997; Elsaleh et al., 2000).

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References

Ainsworth BE, Haskell WL, Leon AS, et al (1993). Compendium of physical activities: classification of energy costs of human physical activities. *Med Sci Sports Exerc*, **25**, 71-80.

Baron JA, Beach M, Mandel JS, et al (1999). Calcium supplements for the prevention of colorectal adenomas. *N Engl J Med*, **340**, 101-7.

Breivik J, Lothe RA, Meling GI, et al (1997). Different genetic pathways to proximal and distal colorectal cancer influenced by sex-related factors. *Int J Cancer*, **74**, 664-9.

Bonithon-Kopp C, Kronborg O, Giacosa A, et al (2000). Calcium and fibre supplementation in prevention of colorectal adenoma recurrence: a randomized intervention trial. *Lancet*, **356**, 1300-6.

Chiu BCH, Ji BT, Dai Q, et al (2003). Dietary factors and risk of colon cancer in Shanghai, China. *Cancer Epidemiol Biomarkers Prev*, **12**, 201-8.

Cho E, Smith-Warner SA, Spiegelman D, et al (2004). Dairy foods, calcium, and colorectal cancer: a pooled analysis of 10 cohort studies. *J Natl Cancer Inst*, **96**, 1015-22.

Elsaleh H, Joseph D, Grieu F, et al (2000). Association of tumour site and sex with survival benefit from adjuvant chemotherapy in colorectal cancer. *Lancet*, **355**, 1745-50.

Flood A, Velie EM, Sinha R, et al (2003). Meat, fat, and their subtypes as risk factors for colorectal cancer in a prospective cohort of women. *Am J Epidemiol*, **158**, 59-68.

Haenszel W, Locke FB, Segi M (1980). A case-control study of large bowel cancer in Japan. *J Natl Cancer Inst*, **64**, 17-22.

Hill M (2001). ECP dietary advice on cancer prevention. *Eur J Cancer Prev*, **10**, 183-9.

Hirayama T (1990). Life-Style and Mortality: a Large-Scale Census-Based Cohort Study in Japan. Karger, Basel.

Hoshiyama Y, Sekine T, Sasaba T (1993). A case-control study of colorectal cancer and its relation to diet, cigarettes, and alcohol consumption in Saitama Prefecture, Japan. *Tohoku J Exp Med*, **171**, 153-65.

Japan Ministry of Health, Labour and Welfare (1999). Annual Report of the National Nutrition Survey, 1997. Daiichi-shuppan, Tokyo.

Kato I, Tominaga S, Matsuura A, et al (1990). A comparative case-control study of colorectal cancer and adenoma. *Jpn J Cancer Res*, **81**, 1101-8.

Kondo R (1975). Epidemiological study on cancer of the colon and the rectum. (II) etiological factors in cancer of the colon and the rectum. *Nagoya Igaku*, **97**, 93-116.

Kono S (1992). Green tea and colon cancer. *Jpn J Cancer Res*, **83**, 669.

Kono S (1996). Dietary factors for gastrointestinal cancers: a worldwide overview. *Gann Monogr Cancer Res*, **44**, 29-39.

Kono S (2004). Secular trend of colon cancer incidence and mortality in relation to fat and meat intake in Japan. *Eur J Cancer Prev*, **13**, 127-32.

Kono S, Ahn Y-O (2000). Vegetables, cereals and colon cancer mortality: long-term trend in Japan. *Eur J Cancer Prev* 9, 363-5.

Kono S, Ikeda M, Tokudome S, Nishizumi M, Kuratsune M (1987). Cigarette smoking, alcohol and cancer mortality: a cohort study of male Japanese physicians. *Jpn J Cancer Res*, **78**, 1323-8.

Kuratsune M, Honda T, Englyst HN, Cummings JH (1986). Dietary fiber in the Japanese diet as investigated in connection with colon cancer risk. *Jpn J Cancer Res*, **77**, 736-8.

Lee D-H, Anderson KE, Harnack LJ, Folsom AR, Jacobs DR Jr (2004). Hem iron, zinc, alcohol consumption, and colon cancer: Iowa Women's Health Study. *J Natl Cancer Inst*, **96**, 403-7.

Le Marchand L, Donlon T, Seifried A, Wilkens LW (2002). Red meat intake, CYP2E1 genetic polymorphisms, and colorectal cancer risk. *Cancer Epidemiol Biomarkers Prev*, **11**, 1019-24.

Le Marchand L, Hankin JH, Wilkens LR, et al (2001). Combined effects of well-done red meat, smoking, and rapid N-acetyltransferase 2 and CYP1A2 phenotypes in increasing colorectal cancer risk. *Cancer Epidemiol Biomarkers Prev*, **10**, 1259-66.

Le Marchand L, Wilkens LR, Hankin, JH, Kolonel LN, Lyu LC (1997). A case-control study of diet and colorectal cancer in a multiethnic population in Hawaii (United States): lipids and foods of animal origin. *Cancer Causes Control*, **8**, 637-48.

Matsuo K, Hamajima N, Hirai T, et al (2002). Methionine synthase reductase gene A66G polymorphism is associated with risk of colorectal cancer. *Asian Pac J Cancer Prev*, **3**, 353-9.

Nagano J, Sudo N, Kaihara C, Shimura M, Kubo C (2001). Validity and reliability of the stress inventory: self-administered questionnaire to assess disease-prone personalities (in Japanese). *Kenko-Shien* (Jpn J Health Promotion), **3**, 107-19.

Nishi M, Yoshida K, Hirata K, Miyake H (1997). Eating habits and colorectal cancer. *Oncol Rep*, **4**, 995-8.

Norat T, Lukanova A, Ferrari P, Riboli E (2002). Meat consumption and colorectal cancer risk: dose-response meta-analysis of epidemiological studies. *Int J Cancer*, **98**, 241-56.

Olson SH (2001). Reported participation in case-control studies: changes over time. *Am J Epidemiol*, **154**, 574-81.

Otani T, Iwasaki M, Yamamoto S, et al (2003). Alcohol consumption, smoking, and subsequent risk of colorectal cancer in middle-aged and elderly Japanese men and women: Japan Public Health Center-based Prospective Study. *Cancer Epidemiol Biomarkers Prev*, **12**, 1492-500.

Parkin DM, Bray F, Ferlay J, Pisani P (2001). Estimating the world cancer burden: GLOBOCAN 2000. *Int J Cancer*, **94**, 153-6.

Parkin DM, Whelan SL, Ferlay J, Raymond L, Young J, eds (1997). Cancer Incidence in Five Continents Vol 7. IARC Scientific Publication No. 143, IARC Press, Lyon.

Parkin DM, Whelan SL, Ferlay J, Teppo L, Thomas DB, eds (2002). Cancer Incidence in Five Continents Vol 8. IARC Scientific Publication No. 155, IARC Press, Lyon.

Sharp L, Little J (2004). Polymorphisms in genes involved in folate metabolism and colorectal neoplasia: a HuGE review. *Am J Epidemiol*, **159**, 423-43.

Shimizu N, Nagata C, Shimizu H, et al (2003). Height, weight, and alcohol consumption in relation to the risk of colorectal cancer in Japan: a prospective study. *Br J Cancer*, **88**, 1038-43.

Slattery ML, Potter J, Caan B, et al (1997). Energy balance and colon cancer beyond physical activity. *Cancer Res*, **57**, 75-80.

Slattery ML, Potter JD, Samowitz W, Schaffer D, Leppert M (1999). Methylenetetrahydrofolate reductase, diet, and risk of colon cancer. *Cancer Epidemiol Biomarkers Prev*, **8**, 513-8.

Tajima K, Tominaga S (1985). Dietary habits and gastro-intestinal cancers: a comparative case-control study of stomach and large intestinal cancers in Nagoya, Japan. *Jpn J Cancer Res*, **76**, 705-16.

Tokudome S, Ikeda M, Tokudome Y, et al (1998). Development of data-based semi-quantitative food frequency questionnaire for dietary studies in middle-aged Japanese. *Jpn J Clin Oncol*, **28**, 679-87.

Toyomura K, Kono S (2002). Soybeans, soy foods, isoflavones and risk of colorectal cancer: a review of experimental and epidemiological data. *Asian Pacific J Cancer Prev*, **3**, 125-32.

Tsubono Y, Takamori S, Kobayashi M, et al (1996). A data-based approach for designing a semiquantitative food frequency questionnaire for a population-based prospective study in Japan. *J Epidemiol*, **6**, 45-53

Yamane T, Nakatani H, Kikuoka N, et al (1996). Inhibitory effects and toxicity of green tea polyphenols for gastrointestinal carcinogenesis. *Cancer*, **77**, 1662-7.

Vineis P, Malats N, Lang M, et al (1999). Metabolic Polymorphisms and Susceptibility to Cancer. IARC Scientific Publication No. 148, IARC Press, Lyon.

Watanabe Y, Tada M, Kawamoto K, et al (1984). A case-control study of cancer of the rectum and the colon. *Nippon Shokakibyo Gakkai Zasshi*, **81**, 185-93.

WHO Consensus Panel (1999). WHO consensus statement on the role of nutrient in colorectal cancer. *Eur J Cancer Prev*, **8**, 57-62.

World Cancer Research Fund and American Institute for Cancer Research (1997). *Food, Nutrition and the Prevention of Cancer: A Global Perspective*. American Institute for Cancer Research, Washington, DC.

Wynder EL, Kajitani T, Ishikawa S, Dodo H, Takano A (1969). Environmental factors of cancer of the colon and rectum. II. Japanese epidemiological data. *Cancer*, **23**, 1210-20.

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Methylenetetrahydrofolate reductase polymorphism, alcohol intake, and risks of colon and rectal cancers in Korea

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Abstract

Several, but not all, studies have reported that a variant genotype of the polymorphism (C677T) of 5,10-methylenetetrahydrofolate reductase (MTHFR), an enzyme in folate metabolism, is associated with a decreased risk of colorectal cancer. A case–control study was conducted to investigate the association of MTHFR polymorphism and heavy alcohol intake to colon and rectal cancer in Korean. Cases were a consecutive series of patients with histologically confirmed, incident colorectal cancer who were admitted to two university hospitals in Seoul, Korea between 1998 and 2000, and controls were selected at the same hospitals. A total of 243 cases (colon 111, rectum 132) and 225 controls were enrolled. While the genotype of MTHFR was not associated with the overall risk of colorectal cancer, increased colon cancer risk was found to be associated with the CT and TT genotypes combined (multivariate odds ratio [OR] 2.01, 95% confidence interval [CI] 1.14–3.53) compared with the wild type. The risk of rectal cancer was found to be, though statistically non-significant, lower in those with the CT and TT genotypes combined (multivariate OR 0.67, 95% CI: 0.43–1.07). Those consuming two or more drinks per day (30 g+/day) had nearly twice the colorectal cancer risk (multivariate OR 1.94, 95% CI 1.03–3.68) of light or non-drinkers (<5 g/day). The present study did not find a reduced risk of colorectal or rectal cancer among those with a variant genotype of the MTHFR polymorphism, but observed rather an increased risk of colon cancer, suggesting that the effects of the MTHFR genotype may differ in populations with different levels of folate intake.

Keywords: Colorectal cancer; Methylenetetrahydrofolate reductase (MTHFR); Alcohol intake; Case–control study

1. Introduction

Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in the regulation of folate metabolism. MTHFR converts 5,10-methylenetetrahydrofolate to

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5-methyltetrahydrofolate which is the major form of folate in blood and a primary methyl donor in the formation of methionine. A common genetic polymorphism of MTHFR (C677T) has recently been the subject of much interest in the etiology of colorectal cancer (CRC). Several studies reported a decreased risk of colorectal cancer in individuals with a homozygous variant of the MTHFR (TT genotype) [1–5]. This effect of the homozygous mutation of MTHFR was hypothesized to be related to quantitative balance of 5,10 methylenetetrahydrofolate [1], which is responsible for both biological methylation and nucleotide synthesis [6]. However, two recent case–control studies showed an increased risk of CRC associated with the TT genotype in Australia [7], especially among the elderly, and in Mexico [8]. No association was found between the MTHFR genotype and CRC in a German study [9].

Alcohol intake has been related to an increased risk of CRC although the increase is relatively small [10,11]. Alcohol is an antagonist of methyl group metabolism, and may contribute to abnormal DNA methylation [12]. Interestingly, a beneficial effect of the MTHFR TT genotype on the risk of CRC was abolished in those with a high alcohol intake [1,2].

On the other hand, it has been also suggested that colon and rectal cancer might have different carcinogenic pathways, based on their genetic and epidemiologic characteristics [13]. In this hospital-based case–control study, we examined the effects of the MTHFR polymorphism, alcohol intake, and of their interaction on the risks of cancers of the colon and rectum in the Korean population.

2. Materials and methods

2.1. Subjects

The cases comprised of a consecutive series of patients, aged 30–79 years, with histologically confirmed incident colorectal adenocarcinomas, who were admitted to two university hospitals in Seoul, Korea between 1998 and 2000. Distinction was made between colon and rectal cancer; the colon included the cecum through to the sigmoid colon, and the rectum included the rectosigmoid junction. A total of 243 patients (colon cancer 111, rectal cancer 132) were recruited. Controls were selected from among patients aged 30–79 years admitted to orthopedics, general surgery, or otorhinolaryngology wards, for a wide spectrum of non-neoplastic conditions, including acute appendicitis, acute otitis media, inguinal hernia, and non-traumatic orthopedic disorder, during the same period as the case admissions; those with a prior history of malignant neoplasms were excluded. A total of 225 controls were identified.

2.2. Data collection

A trained nurse interviewer ascertained smoking habit, alcohol intake, diet, and other lifestyle factors using a structured questionnaire. As regards alcohol intake, participants reported the consumption frequency (two or more/day, daily, 5–6/week, 2–4/week, 1/week, 1–3/month, and rarely/never) and amount of each type of beverage (soju, beer, wine, liquor) consumed during the year before the onset of symptom. For individual alcoholic beverages, daily ethanol intake in grams was calculated based on the ethanol content of the beverage. Total ethanol intake was calculated, and categorized into three levels (<5, 5–29, and 30+ g/day); 30 g of ethanol roughly corresponds two drinks in Western countries. They also completed a validated, comprehensive food frequency questionnaire containing 80 food items commonly eaten in Korea [14]. Total energy intake was calculated using the sixth version of the Korean food composition table [15]. Red meat intake was categorized into quartiles based on the frequency distribution of the control subjects. Vigorous activity was defined as participation in activities with a metabolic equivalent [MET] of 6.0 or more, such as bicycling, jogging, swimming, mountain climbing or aerobics, based on a compendium of physical activities, as proposed by Ainsworth et al. [16]. Smoking history was categorized as none, past or current.

2.3. MTHFR genotyping

Venous blood was taken at the time of interview with written informed consent. The buffy coat was kept at −70 °C at the Seoul National University, and frozen materials were transported to a laboratory at Fukuoka University for genetic analysis. DNA was

extracted from the buffy coat using a commercial kit (QIAGEN GmbH, Hilden, German). MTHFR genotype was determined by one of the authors (E.T) by PCR-RFLP, as described by Fross et al. [17]. PCR was performed in a reaction mixture of 50 μl containing 1.0 μg of template DNA. After an initial denaturation at 95 °C for 10 min, 35 cycles of PCR were performed (1 min at 95 °C, 1 min at 68 °C and 1 min at 72 °C) with a final extension at 72 °C for 7 min. The reaction stopped by chilling to 4 °C. The PCR products were then digested with *Hin*fI, which cleaves the 198-bp PCR product into two fragments of 175 and 23 bp when the C677T mutation is present. The digested PCR products were electrophoresed in 9% acrylamide gel, and visualized by ethidium bromide.

2.4. Statistical analysis

The associations of MTHFR genotype and alcohol intake with the risk of colorectal cancers were examined by multiple logistic regression analysis in terms of odds ratios (ORs) and 95% confidence intervals (CIs), allowing for the effect of potential covariates such as age, sex, total energy intake, family history of colorectal cancer, body mass index, smoking, vigorous physical activity, red meat intake, and alcohol drinking. For the MTHFR genotype, the effects of heterozygous (CT) and homozygous variant (TT) polymorphism (or of CT+TT combined polymorphism) were compared with that of the wild type (CC). We examined whether the MHHFR genotype modified the associations between alcohol intake and the colorectal cancer risks by including an interaction term between the MTHFR genotype (CT+TT versus CC) and total alcohol intake (three-level ordinal variable) in the multivariate model. Statistical significance was accepted if the two-sided *P*-value was less than 0.05 or if the 95% CI did not include unity. All statistical analyses were performed using SAS [18].

3. Results

The demographic and lifestyle characteristics of the cases and controls are shown in Table 1. No significant differences were found in the distributions of age, sex, body mass index, exercise history or the use of multivitamins between the cases and controls. Cases were found to have a family history of colorectal cancer among first degree relatives more frequently ($P=0.01$), to consume more alcohol ($P<0.01$), to be more likely to be past/current smokers ($P=0.02$), and to eat red meat more frequently ($P=0.01$) than the controls. The frequency of the T allele of MTHFR was similar in case and control subject (39.5 versus 38.9%, respectively, $P=0.93$), and the distribution of MTHFR genotypes among the controls was in Hardy–Weinberg equilibrium.

The frequency of the CC, CT, and TT genotypes were 36.9, 48.4 and 14.7% among the controls and 35.4, 50.2 and 14.4% among the cases, respectively (Table 2). The overall risk of colorectal cancer did not appreciably vary with MTHFR genotype whether adjustment was made for confounding factors or not. As compared with those who had the CC genotype,

Table 1
General characteristics of study subjects of colorectal cancer study

Factor	Cases ($n=243$)	Controls ($n=225$)	*P* value
Age (years)	56 (11)	55 (11)	0.51
Sex (female, %)	44	52	0.08
Family history of cancer[a] (%)	7.0	1.3	<0.01
Body mass index (kg/m^2)	23.8 (2.9)	23.8 (3.2)	0.86
Vigorous exercise (%)	28.9	31.7	0.51
Alcohol (g/day, %)			
<5	57.2	68.0	
5–29	19.3	20.0	
30+	23.5	12.0	<0.01
Smoking (%)			
None	54.7	66.2	
Past	9.9	5.3	
Current	35.4	28.4	0.02
Regular use of multivitamin (%)	19.8	21.8	0.59
Red meat intake (mean servings/week)	3.1 (2.6)	2.5 (2.2)	0.01
T allele of MTHFR (%)	39.5	38.9	0.93

Values are means and standard deviations in parentheses unless otherwise specified.
[a] Colorectal cancer among the first-degree family members.

Table 2
Adjusted odds ratio (OR) and 95% confidence intervals (CI) of colorectal cancer according to methylenetetrahydrofolate reductase (MTHFR) genotypes and alcohol intake

Factor	No. of cases (%)	No. of controls (%)	Age- and sex-adjusted OR (95% CI)	Multivariate-adjusted OR (95% CI)[a]
MTHFR				
CC	86 (35.4)	83 (36.9)	1.00 (ref)	1.00 (ref)
CT	122 (50.2)	109 (48.4)	1.08 (0.72–1.60)	1.07 (0.70–1.63)
TT	35 (14.4)	33 (14.7)	1.02 (0.58–1.79)	0.90 (0.49–1.64)
Alcohol (g/day)				
<5	139 (57.2)	153 (68.0)	1.00 (ref)	1.00 (ref)
5–29	47 (19.3)	45 (20.0)	1.15 (0.72–1.83)	0.94 (0.53–1.68)
30+	57 (23.5)	27 (12.0)	2.33 (1.39–3.88)	1.94 (1.03–3.68)

[a] Adjusted for age, sex, total energy intake, family history of colorectal cancer, body mass index, smoking, vigorous physical activity, red meat intake, and the two listed factors.

those who were heterozygous (CT) had a multivariate OR of 1.07 (95% CI, 0.70–1.63) and those with a homozygous variant (TT) had a risk of 0.90 (95% CI 0.49–1.64). No clear difference was observed in terms of the risk of CRC in relation to the MTHFR polymorphism according to age group (<60 versus 60+ years old, data not shown). However, a high consumption of alcohol was found to be associated with a statistically significant increase in the risk of colorectal cancer, regardless of covariate adjustment (Table 2). Those consuming two or more drinks per day (30 g+/day) had nearly twice the colorectal cancer risk (multivariate OR 1.94, 95% CI 1.03–3.68) of light or non-drinkers (<5 g/day).

When colon and rectal cancer were examined separately (Table 3), an approximately two-fold increase in the odds of colon cancer was observed among individuals with the CT and TT genotypes each as compared with those with the CC genotype. The adjusted ORs of rectal cancer for the CT and TT genotypes were lower than unity. The adjusted ORs of colon and rectal cancer for the CT and TT genotypes combined as compared with the CC genotype were 2.01 (95% CI 1.14–3.53) and 0.67 (95% CI 0.43–1.07), respectively. A high intake of alcohol was found to be associated with a statistically significant increase in the risk of colon cancer, and with a non-significantly elevated risk of rectal cancer.

The interaction of the MTHFR genotype and alcohol drinking was examined in relation to the risks of colorectal, colon and rectal cancer separately (Table 4). In this analysis, the CT and TT genotypes were combined, because their associated risks were

Table 3
Adjusted odds ratios (OR) and 95% confidence intervals (CI) of colon and rectal cancer according to methylenetetrahydrofolate reductase (MTHFR) genotypes and alcohol intake

Factor	Colon cancer			Rectal cancer		
	No.	OR (95% CI)[a]		No.	OR (95% CI)	
MTHFR						
CC	24	1.00		62	1.00	
CT	67	2.04 (1.13–3.67)		55	0.71 (0.43–1.15)	
TT	20	1.85 (0.85–4.01)	2.01 (1.14–3.53)	15	0.57 (0.27–1.19)	0.67 (0.43–1.07)
Alcohol (g/day)						
<5	58	1.00		81	1.00	
5–29	23	1.24 (0.58–2.67)		24	0.74 (0.37–1.48)	
30+	30	2.72 (1.21–6.11)		27	1.42 (0.67–3.01)	

[a] Adjusted for age, sex, total energy intake, family history of colorectal cancer, body mass index, smoking, vigorous physical activity, red meat intake, and the other two of the listed factors.

Table 4
Risks of colorectal cancer, colon cancer, and rectal cancer according to methylenetetrahydrofolate reductase (MTHFR) genotype and alcohol intake in combination

MTHFR	Alcohol (g/day)			*P* for trend
	<5	5–29	30+	
Colorectal cancer				
CC	57/52[a]	18/14	8/20	
	1.00 (ref)	0.70 (0.29–1.68)	1.98 (0.73–5.39)	0.26
CT+TT	96/87	27/33	19/37	
	0.96 (0.59–1.57)	1.05 (0.51–2.16)	1.75 (0.81–3.77)	0.19
Colon cancer				
CC	57/13	18/4	8/7	
	1.00 (ref)	0.95 (0.25–3.66)	3.54 (0.92–13.6)	0.08
CT+TT	96/45	27/19	19/23	
	1.99 (0.96–4.11)	2.76 (1.04–7.36)	4.95 (1.82–13.5)	<0.001
Rectal cancer				
CC	57/39	18/10	8/13	
	1.00 (ref)	0.57 (0.21–1.53)	1.51 (0.51–4.46)	0.43
CT+TT	96/42	27/14	19/14	
	0.63 (0.36–1.12)	0.56 (0.24–1.33)	0.85 (0.34–2.14)	0.77

[a] Upper numbers are numbers of controls/cases, and lower numbers are adjusted odds ratios and 95% confidence intervals in parentheses. Adjustment was done for age, sex, family history of colorectal cancer, body mass index, smoking, total energy intake, vigorous physical activity, and red meat.

similar. No clear interaction between the MTHFR genotype and alcohol intake was observed in terms of the risk of colorectal cancer ($P=0.87$), colon cancer ($P=0.74$), or rectal cancer ($P=0.91$). Alcohol drinking was found to be associated with an increased risk of colon cancer in subjects with the CC genotype and in those with CT or TT genotype, although the association with the CC genotype marginally failed to reach statistical significance. No measurable increase in the risk of rectal cancer could be attributed to alcohol intake for either genotype group.

4. Discussion

The overall risk of colorectal cancer was found to be unrelated to the MTHFR genotype, but the T allele was found to be associated with an increased risk of colon cancer and with a somewhat decreased risk of rectal cancer. Heavy drinkers had nearly twice the risk of colorectal cancer. The relations between alcohol drinking and colorectal, colon and rectal cancer were found not to differ by MTHFR genotype in this study. A unique finding in the present study was that an increased risk of colon cancer was contrasted by a decreased risk of rectal cancer, among those with the T allele.

Previous studies in the United States have shown a protective association between the homozygous variant of the MTHFR genotype and colon [3] or colorectal cancer [1,2,4]. Chen [1] and Ma [2] have also reported that a protective association with the TT genotype disappeared when folate or methionine intake was low and alcohol intake was high. We could not evaluate the effects of folate and methionine on the association between the MTHFR genotypes and CRC risk due to a lack of reliable information on the consumptions of these micronutrients. Moreover, folate intake may be much lower in Korea than in the United States. According to a limited number of studies [19,20], the average daily intake of folate in Korea is far less than 200 μg/day. And, it was reported in the third NHANES [21] that the mean daily intake of folate was 317 μg for men and 236 μg for women in the general population in the United States. Our results are in agreement with recent findings in Australia and Mexico. In a case–control study in Australia, Shannon et al. [7] demonstrated that the TT genotype is more frequently found in patients with colorectal cancer, compared with age-matched

controls, especially among the elderly, whose folate intake was expectedly low. They suggested that the TT genotype is an additional risk factor for CRC when the methyl metabolism is disturbed. Similarly, an approximately two-fold increased risk of colorectal cancer was observed among those with the CT or TT genotype versus those with the CC genotype, in a Mexican case–control study. [8]. These studies, together with the present study, suggest that the effect of the MTHFR genotype depends upon the population and folate intake. This may also explain the recent findings that the MTHFR 677TT genotype is associated with an increased risk of esophageal [22] and stomach cancer [23] in China.

The observed differential associations between the MTHFR genotype and colon and rectal cancer may be due to chance in the subgroup analysis, and caution should be exercised in the interpretation of these findings. It has been, however, suggested that colorectal cancer may not be one homogenous disease entity by subsite of origin [13]. Colon to rectal cancer incidence rate ratios are higher in populations with a high colorectal cancer risk, while these ratios are correspondingly lower in most low risk countries [24]. Moreover, these rate ratios tend to increase as the overall incidence of colorectal cancer increases, and this relation was particularly obvious in Japan [25]. It has been well documented that colon and rectal cancers have different male-to-female incidence ratios, and that the male excess is more pronounced from the colon to the rectum [26]. Further, molecular alterations, such as K-ras and p53 mutations, were found to be more frequent in the distal part of the large bowel, while microsatellite instability was almost exclusively associated with the proximal CRC [27].

Shannon et al. [7] found that the homozygous variant genotype of MTHFR appeared to predispose CRC with microsatellite instability. This MSI was found exclusively in the proximal CRC [27,28]. This may provide another explanation why we observed an increased risk of colon cancer in those with the TT genotypes, but not in those with rectal cancer.

A high alcohol intake was found to be positively associated with the risk of colon cancer, but was not found to be associated with the risk of rectal cancer. However, this apparent difference could have been due to random variation, as indicated by the overlapping of confidence intervals. The overall risk of colorectal cancer was significantly higher for those consuming 30 g or more of alcohol per day. Moreover, it was suggested in a qualitative review [29] and in a quantitative meta-analysis [10] that alcohol is an independent risk factor of colorectal cancer. In a recent pooled analysis of eight prospective cohort studies conducted in the Western countries, a 24% increase in the risk of colorectal cancer was found for those consuming 30 g of alcohol or more per day, with no appreciable difference between colon and rectal cancers [30].

Several mechanisms may be proposed to explain this increased risk of colorectal cancer with respect to alcohol intake. In rats fed a diet with a normal folate content, alcohol administration increased intracolonic acetaldehyde levels and decreased significantly colonic mucosal folate levels [31], possibly due to the cleavage of folate by acetaldehyde [32]. Moreover, elevated alcohol intake may be related to delayed DNA repair [33], the activation of liver procarcinogen by the induction of cytochrome p-450 enzymes [33], or a change in bile acid composition [29].

In conclusion, the present case–control study in Korea did not find a reduced risk of colorectal or rectal cancer among those with a variant genotype of the MTHFR polymorphism, but observed rather an increased risk of colon cancer, suggesting that the effects of the MTHFR genotype may differ in populations with different levels of folate intake.

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References

[1] J. Chen, E. Giovannucci, K. Kelsey, E.B. Rimm, M.J. Stampfer, G.A. Colditz, et al., A methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer, Cancer Res. 56 (1996) 4862–4864.

[2] J. Ma, M.J. Stampfer, E. Giovannucci, C. Artigas, D.J. Hunter, C. Fuchs, et al., Methylenetetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer, Cancer Res. 57 (1997) 1098–1102.

[3] M.L. Slattery, J.D. Potter, W. Samowitz, D. Schaffer, M. Leppert, Methylenetetrahydrofolate reductase, diet, and risk of colon cancer, Cancer Epidemiol. Biomarkers Prev. 8 (1999) 513–518.
[4] L. Le Marchand, T. Donlon, J.H. Hankin, L.N. Kolonel, L.R. Wilkens, A. Seifried, B-vitamin intake, metabolic genes, and colorectal cancer risk (United States), Cancer Causes Control 13 (2002) 239–248.
[5] G. Toffoli, R. Gafa, A. Russo, G. Lanza, R. Dolcetti, F. Sartor, et al., Methylenetetrahydrofolate reductase 677 C→T polymorphism and risk of proximal colon cancer in north Italy, Clin. Cancer Res. 9 (2003) 743–748.
[6] Y.I. Kim, Folate and carcinogenesis: evidence, mechanisms, and implications, J. Nutr. Biochem. 10 (1999) 66–88.
[7] B. Shannon, S. Gnanasampanthan, J. Beilby, B. Iacopetta, A polymorphism in the methylenetetrahydrofolate reductase gene predisposes to colorectal cancers with microsatellite instability, Gut 50 (2002) 520–524.
[8] I. Delgado-Enciso, S.G. Martinez-Garza, A. Rojas-Martinez, R. Ortiz-Lopez, F. Bosques-Padilla, A.L. Calderon-Garciduenas, et al., 677T mutation of the MTHFR gene in adenomas and colorectal cancer in a population sample from the Northeastern Mexico. Preliminary results, Rev. Gastroenterol. Mex. 66 (2001) 32–37.
[9] J. Plaschke, U. Schwanebeck, S. Pistorius, H.D. Saeger, H.K. Schackert, Methylenetetrahydrofolate reductase polymorphisms and risk of sporadic and hereditary colorectal cancer with or without microsatellite instability, Cancer Lett. 191 (2003) 179–185.
[10] M.P. Longnecker, M.J. Orza, M.E. Adams, J. Vioque, T.C. Chalmers, A meta-analysis of alcoholic beverage consumption in relation to risk of colorectal cancer, Cancer Causes Control 1 (1990) 59–68.
[11] J.D. Potter, M.L. Slattery, R.M. Bostick, S.M. Gapstur, Colon cancer: a review of the epidemiology, Epidemiol. Rev. 15 (1993) 499–545.
[12] Anonymous, Folate, alcohol, methionine, and colon cancer risk: is there a unifying theme? Nutr. Rev. 52 (1994) 18–20.
[13] J.A. Bufill, Colorectal cancer: evidence for distinct genetic categories based on proximal or distal tumor location, Ann. Int. Med. 113 (1990) 779–788.
[14] M.K. Kim, S.S. Lee, Y.O. Ahn, Reproducibility and validity of a self-administered semiquantitative food frequency questionnaire among middle-aged men in Seoul, Korean J. Community Nutr. 1 (1998) 376–394.
[15] K.N. Society, Recommended Daily Average Among Koreans, Seoul, 1995.
[16] B. Ainsworth, W. Haskell, A. Leon, D. Jacobs Jr., H. Montoye, J. Sallis, et al., Compendium of physical activities: classification of energy costs of human physical activities, Med. Sci. Sports Exerc. 25 (1993) 71–80.
[17] P. Frosst, H.J. Blom, R. Milos, P. Goyette, C.A. Sheppard, R.G. Matthews, et al., Candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase (letter), Nat. Genet. 10 (1995) 111–113.
[18] SAS Institute Inc., Version 6.12, SAS Institute, Inc., Cary, NC, 1996.
[19] T. Hyun, Y. Han, Y. Lim, Blood folate level determined by a microplate reader and folate intake measured by a weighed food record, Korean J. Community Nutr. 4 (1999) 512–520.
[20] H. Lim, H. Jin, J. Lee, Dietary intakes and status of folate in Korean Women of child-bearing potential, Korean J. Nutr. 33 (2000) 296–303.
[21] K. Alaimo, M. McDowell, R. Briefel, A. Bischof, C. Caughman, C. Loria, et al., Dietary intake of vitamins, minerals, and filber of persons ages 2 months and over in the United States: Third National Health and Nutrition Examination Survey, Phase I, 1988–91, in: Vital and Health Statistics of the Center For Disease Control and Prevention, 1994, p. 10.
[22] C. Song, D. Xing, W. Tan, Q. Wei, D. Lin, Methylenetetrahydrofolate reductase polymorphisms increase risk of esophageal squamous cell carcinoma in a Chinese population, Cancer Res. 61 (2001) 3272–3275.
[23] X. Miao, D. Xing, W. Tan, J. Qi, W. Lu, D. Lin, Susceptibility to gastric cardia adenocarcinoma and genetic polymorphisms in methylenetetrahydrofolate reductase in an at-risk Chinese population, Cancer Epidemiol. Biomarkers Prev. 11 (2002) 1454–1458.
[24] R.G. Ziegler, S.S. Devesa, J.F. Fraumeni Jr., Epidemiologic patterns of colorectal cancer, in: V.T. DeVita, S. Hellman, S.A. Rosenberg (Eds.), Important Advances in Oncology, Lippincott, Philadelphia, PA, 1986, pp. 209–232.
[25] K. Tamura, S. Ishiguro, A. Munakata, Y. Yoshida, S. Nakaji, K. Sugawara, Annual changes in colorectal carcinoma incidence in Japan. Analysis of survey data on incidence in Aomori Prefecture, Cancer 78 (1996) 1187–1194.
[26] S.S. Devesa, W.H. Chow, Variation in colorectal cancer incidence in the United States by subsite of origin, Cancer 71 (1993) 3819–3826.
[27] J. Breivik, R.A. Lothe, G.I. Meling, T.O. Rognum, A.L. Borresen-Dale, G. Gaudernack, Different genetic pathways to proximal and distal colorectal cancer influenced by sex-related factors, Int. J. Cancer 74 (1997) 664–669.
[28] S.N. Thibodeau, G. Bren, D. Schaid, Microsatellite instability in cancer of the proximal colon, Science 260 (1993) 816–819.
[29] G.A. Kune, L. Vitetta, Alcohol consumption and the etiology of colorectal cancer: a review of the scientific evidence from 1957 to 1991, Nutr. Cancer 18 (1992) 97–111.
[30] E. Cho, S.A. Smith-Warner, J. Ritz, P.A. van den Brandt, G.A. Colditz, A.R. Folsom, et al., Alcohol and colorectal cancer: a pooled analysis of 8 cohort studies, Ann. Intern. Med. 140 (2004) 603–613.
[31] N. Homann, J. Tillonen, M. Salaspuro, Microbially produced acetaldehyde from ethanol may increase the risk of colon cancer via folate deficiency, Int. J. Cancer 86 (2000) 169–173.
[32] M. Salaspuro, Bacteriocolonic pathway for ethanol oxidation: characteristics and implications, Ann. Med. 28 (1996) 195–200.
[33] H.K. Seitz, G. Poschl, U.A. Simanowski, Alcohol and cancer, Recent Dev. Alcohol 14 (1998) 67–95.

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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1. Lucock M. Folic acid: nutritional biochemistry, molecular biology and role in disease processes. *Mol Genet Metab* 2000; **71**: 121–38.
2. Sharp L, Little J. Polymorphisms in genes involved in folate metabolism and colorectal neoplasia: a HuGE review. *Am J Epidemiol* 2004; **159**: 423–43.
3. Blount BC, Mack MM, Wehr CM, MacGregor JT, Hiatt RA, Wang G, Wickramasinghe SN, Everson RB, Ames BN. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. *Proc Natl Acad Sci USA* 1997; **94**: 3290–5.
4. Duthie SJ. Folic acid deficiency and cancer: mechanisms of DNA instability. *Br Med Bull* 1999; **55**: 578–92.
5. Duthie SJ, Narayanan S, Blum S, Pirie L, Brand G. Folate deficiency *in vitro* induces uracil misincorporation and DNA hypomethylation and inhibits DNA excision repair in immortalized normal colon epithelial cells. *Nutr Cancer* 2000; **37**: 245–51.
6. Laird PW, Jaenisch R. The role of DNA methylation in cancer genetic and epigenetics. *Annu Rev Genet* 1996; **30**: 441–64.
7. Toyota M, Ahuja N, Ohe-Toyota M, Herman JG, Baylin SB, Issa JP. CpG island methylator phenotype in colorectal cancer. *Proc Natl Acad Sci USA* 1999; **96**: 8681–6.
8. van Rijnsoever M, Grieu F, Elsaleh H, Joseph D, Iacopetta B. Characterisation of colorectal cancers showing hypermethylation at multiple CpG islands. *Gut* 2002; **51**: 797–802.
9. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M, Kluijtmans LA, van den Heuvel LP *et al.* A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet* 1995; **10**: 111–3.
10. van der Put NM, Gabreels F, Stevens EM, Smeitink JA, Trijbels FJ, Eskes TK, van den Heuvel LP, Blom HJ. A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? *Am J Hum Genet* 1998; **62**: 1044–51.
11. Chen J, Giovannucci E, Kelsey K, Rimm EB, Stampfer MJ, Colditz GA, Spiegelman D, Willett WC, Hunter DJ. A methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer. *Cancer Res* 1996; **56**: 4862–4.
12. Ma J, Stampfer MJ, Giovannucci E, Artigas C, Hunter DJ, Fuchs C, Willett WC, Selhub J, Hennekens CH, Rozen R. Methylenetetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer. *Cancer Res* 1997; **57**: 1098–102.
13. Slattery ML, Potter JD, Samowitz W, Schaffer D, Leppert M. Methylenetetrahydrofolate reductase, diet, and risk of colon cancer. *Cancer Epidemiol Biomarkers Prev* 1999; **8**: 513–8.
14. Le Marchand L, Donlon T, Hankin JH, Kolonel LN, Wilkens LR, Seifried A. B-Vitamin intake, metabolic genes, and colorectal cancer risk (United States). *Cancer Causes Control* 2002; **13**: 239–48.
15. Keku T, Millikan R, Worley K, Winkel S, Eaton A, Biscocho L, Martin C, Sandler R. 5,10-Methylenetetrahydrofolate reductase, codon 677 and 1298 polymorphisms and colon cancer in African Americans and whites. *Cancer Epidemiol Biomarkers Prev* 2002; **11**: 1611–20.
16. Plaschke J, Schwanebeck U, Pistorius S, Saeger HD, Schackert HK. Methylenetetrahydrofolate reductase polymorphisms and risk of sporadic and hereditary colorectal cancer with or without microsatellite instability. *Cancer Lett* 2003; **191**: 179–85.
17. Sachse C, Smith G, Wilkie MJ, Barrett JH, Waxman R, Sullivan F, Forman D, Bishop DT, Wolf CR. A pharmacogenetic study to investigate the role of dietary carcinogens in the etiology of colorectal cancer. *Carcinogenesis* 2002; **23**: 1839–49.
18. Toffoli G, Gafa R, Russo A, Lanza G, Dolcetti R, Sartor F, Libra M, Viel A, Boiocchi M. Methylenetetrahydrofolate reductase 677C→T polymorphism and risk of proximal colon cancer in north Italy. *Clin Cancer Res* 2003; **9**: 743–8.
19. Shannon B, Gnanasampanthan S, Beilby J, Iacopetta B. A polymorphism in the methylenetetrahydrofolate reductase gene predisposes to colorectal cancers with microsatellite instability. *Gut* 2002; **50**: 520–4.
20. Park KS, Mok JW, Kim JC. The 677C>T mutation in 5,10-methylenetet-

rahydrofolate reductase and colorectal cancer risk. *Genet Test* 1999; **3**: 233–6.
21. Delgado-Enciso I, Martinez-Garza SG, Rojas-Martinez A, Ortiz-Lopez R, Bosques-Padilla F, Calderon-Garciduenas AL, Zarate-Gomez M, Barrera-Saldana HA. 677T mutation of the MTHFR gene in adenomas and colorectal cancer in a population sample from the Northeastern Mexico. Preliminary results. *Rev Gastroenterol Mex* 2001; **66**: 32–7.
22. Chen J, Ma J, Stampfer MJ, Palomeque C, Selhub J, Hunter DJ. Linkage disequilibrium between the 677C>T and 1298A>C polymorphisms in human methylenetetrahydrofolate reductase gene and their contributions to risk of colorectal cancer. *Pharmacogenetics* 2002; **12**: 339–42.
23. Halsted CH, Villanueva JA, Devlin AM, Chandler CJ. Metabolic interactions of alcohol and folate. *J Nutr* 2002; **132**: 2367S–72S.
24. Parkin DM, Whelan SL, Ferlay J, Raymond L, Young J (eds). Cancer incidence in five continents. vol VII. Lyon: IARC; 1998.
25. Shaw S, Jayatilleke E, Herbert V, Colman N. Cleavage of folates during ethanol metabolism. Role of acetaldehyde/xanthine oxidase-generated superoxide. *Biochem J* 1989; **257**: 277–80.
26. Bailey LB. Folate, methyl-related nutrients, alcohol, and the MTHFR 677C→T polymorphism affect cancer risk: intake recommendations. *J Nutr* 2003; **133**: 3748S–58S.
27. Giovannucci E, Chen J, Smith-Warner SA, Rim EB, Fuchs CS, Palomeque C, Willett W, Hunter DJ. Methylenetetrahydrofolate reductase, alcohol dehydrogenase, diet, and risk of colorectal adenomas. *Cancer Epidemiol Biomarkers Prev* 2003; **12**: 970–9.
28. Richman S, Adlard J. Left and right sided large bowel cancer. *BMJ* 2002; **324**: 931–2.
29. Thibodeau SN, Bren G, Schaid D. Microsatellite instability in cancer of the proximal colon. *Science* 1993; **260**: 816–9.
30. Breivik J, Lothe RA, Meling GI, Rognum TO, Borresen-Dale AL, Gaudernack G. Different genetic pathways to proximal and distal colorectal cancer influenced by sex-related factors. *Int J Cancer* 1997; **74**: 664–9.
31. Soong R, Powell B, Elsaleh H, Gnanasampanthan G, Smith DR, Goh HS, Joseph D, Iacopetta B. Prognostic significance of TP53 gene mutation in 995 cases of colorectal carcinoma. Influence of tumour site, stage, adjuvant chemotherapy and type of mutation. *Eur J Cancer* 2000; **36**: 2053–60.
32. Elsaleh H, Joseph D, Grieu F, Zeps N, Spry N, Iacopetta B. Association of tumour site and sex with survival benefit from adjuvant chemotherapy in colorectal cancer. *Lancet* 2000; **355**: 1745–50.
33. Ogino S, Wilson RB. Genotype and haplotype distributions of MTHFR677C>T and 1298A>C single nucleotide polymorphisms: a meta-analysis. *J Hum Genet* 2003; **48**: 1–7.
34. Yoshimura K, Hanaoka T, Ohnami S, Ohnami S, Kohno T, Liu Y, Yoshida T, Sakamoto H, Tsugane S. Allele frequencies of single nucleotide polymorphisms (SNPs) in 40 candidate genes for gene-environment studies on cancer: data from population-based Japanese random samples. *J Hum Genet* 2003; **48**: 654–8.
35. Davey Smith G, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol* 2003; **32**: 1–22.

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Diabetologia

Coffee consumption and glucose tolerance status in middle-aged Japanese men

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Abstract

Aims/hypothesis. Several studies have reported that coffee has a protective effect against the development of type 2 diabetes. However, few of these studies used the standard glucose tolerance test to diagnose type 2 diabetes. The aim of this study was to investigate the relationship between coffee and green tea consumption and glucose tolerance status as determined using a 75-g OGTT.

Methods. We performed a cross-sectional study of 3224 male officials of the self-defence forces. Glucose tolerance status was determined in accordance with the 1998 World Health Organization criteria, and average intakes of coffee and green tea over the previous year were assessed by a self-administered questionnaire. The figures obtained were adjusted for BMI, physical activity and other factors.

Results. A total of 1130 men were identified as having glucose intolerance (IFG, IGT or type 2 diabetes). Compared with those who did not consume coffee on a daily basis, fasting and 2-h post-load plasma glucose levels were 1.5% and 4.3% lower in those who drank 5 cups of coffee or more per day respectively. The adjusted odds ratios of glucose intolerance for categories of <1, 1–2, 3–4 and ≥5 cups of coffee per day were 1.0 (referent), 0.8 (95% CI 0.6–1.0), 0.7 (95% CI 0.6–0.9) and 0.7 (95% CI 0.5–0.9) respectively (p=0.0001 for trend). No clear association was observed between green tea drinking and glucose tolerance status.

Conclusions/interpretation. Coffee consumption may inhibit postprandial hyperglycaemia and thereby protect against the development of type 2 diabetes mellitus.

Keywords Type 2 diabetes · Coffee · Cross-sectional study · Middle-aged · Japanese · Men.

Introduction

Type 2 diabetes has become a global health burden; worldwide, the number of people with type 2 diabetes was approximately 135 million in 1995, and this figure is predicted to rise above 300 million by 2025 [1]. In Japan, it is estimated that nearly 7 million individuals suffer from type 2 diabetes, and that another 7 million have a pre-diabetic condition [2]. Of the lifestyle factors associated with the risk of type 2 diabetes, obesity and physical inactivity are the two most important factors involved in the development of the disease [3]. Additionally, it has been suggested that Japanese individuals may have a higher genetic susceptibility to type 2 diabetes [4, 5].

Recent epidemiological studies have suggested a possible protective effect of coffee against type 2 diabetes. A prospective study performed in the Netherlands reported that coffee drinking was associated with a decreased risk of type 2 diabetes [6]. This finding has since been replicated in several follow-up and cross-sectional studies [7, 8, 9, 10, 11]. However, a

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Abbreviations: MET, metabolic equivalents · SDF, self-defence forces

health examination survey of the Finnish population [12] and a population-based study of Pima Indians [13] failed to observe a protective effect of coffee. Only two of these studies used the standard glucose tolerance test to diagnose type 2 diabetes [11, 13]. The aim of the present study was to investigate the relationship between daily intakes of coffee and green tea and glucose tolerance status as determined by a 75-g OGTT in middle-aged Japanese men.

Subjects and methods

Study population. Subjects were male officials in the self-defence forces (SDF) who underwent a pre-retirement health examination between January 1997 and March 2002 at the Self-Defense Forces Fukuoka Hospital and the Self-Defense Forces Kumamoto Hospital; these two hospitals cover the Kyushu district. All officials retiring from the SDF receive a pre-retirement health examination as part of a nationwide programme which offers a comprehensive medical examination. Details of the health examination have been described elsewhere [14, 15, 16, 17].

We consecutively recruited 3413 men aged 46–59 years, 3224 of whom were included in the present study. Of the remainder, five men refused to participate in the study and 184 men were excluded for the following reasons: endocrine diseases (n=15), chronic pancreatitis (n=9), chronic hepatitis or liver cirrhosis (n=79), use of steroids (n=9), past history of gastrectomy (n=57) and missing information as regards covariates under study (n=20). Some of the men met two or more of the exclusion criteria. There were 17 men whose glucose tolerance status was not determined.

The study was approved by the Ethics Committee of the Kyushu University Faculty of Medical Sciences. All study subjects gave written informed consent prior to their participation in the study.

Glucose and other measurements. Over a 5-day admission period, routine medical tests and examinations included a 75-g OGTT. After an overnight fast, venous blood was drawn for measurement of plasma glucose before and 2 h after the oral glucose load. Plasma glucose levels were determined by the glucose oxidase method using commercial reagents (Shino Test, Tokyo, Japan). Subjects were classified as having normal glucose tolerance, IFG, IGT, or type 2 diabetes in accordance with the World Health Organization (WHO) diagnostic criteria, as revised in 1998 [18]. Men with a history of dietary or drug treatment for type 2 diabetes were regarded as having known type 2 diabetes, irrespective of their glucose levels. Body weight and height were recorded, and BMI (kg/m^2) was calculated as a measure of obesity.

Assessment of coffee and green tea consumption and lifestyle characteristics. A self-administered questionnaire was used to ascertain coffee and green tea consumptions, smoking habits, alcohol use, leisure-time physical activity and other lifestyle characteristics.

Average weekly frequencies of coffee and green tea drinking over the previous year were obtained. Those drinking coffee and green tea on a daily basis reported the number of cups of each consumed per day. In a validation study based on the 28-day diet record over the past year [19], the estimated intake of each beverage showed good agreement between the two methods. The Spearman rank correlation coefficients for coffee and green tea were 0.75 and 0.64 respectively. Caffeine ingestion was estimated using the published caffeine concentrations in coffee (0.04%) and green tea (0.02%) [20]. An average serving of one cup of coffee was estimated to be equal to 150 ml, while an average serving of one cup of green tea was estimated to be equal to 100 ml. Coffee accounted for the majority (66%) of caffeine intake. Ever-smokers were defined as individuals who had ever smoked one cigarette or more per day for at least 1 year or longer. The cumulative exposure of ever-smokers to cigarette smoking was expressed as cigarette-years, the average number of cigarettes smoked per day multiplied by years of smoking. Alcohol drinkers were defined as those who had consumed alcohol once or more per week over a period of 1 year or longer. Past-drinkers were distinguished from lifelong non-drinkers. Alcohol consumption was calculated for current drinkers based on the reported frequencies of consumption of five different alcoholic beverages (sake, shochu, beer, whisky [including brandy] and wine) and amount consumed per occasion over the previous year. Questions on leisure-time physical activity were slightly changed in April 1999. Prior to the revision, the subjects were first asked about their average frequency of regular participation in recreational exercise and sport over the previous year using a closed-ended question (none, 1–2, 3–4, 5–6 times per week or daily). If the subjects participated in recreational physical activity once per week or more frequently, they reported the type of regular exercise or sport and the time spent per occasion. Up to three types of regular exercises were recorded. In the revised questionnaire, the subjects were first asked whether they had regularly participated in recreational activity (one or more times per week) during the previous year. Those who had regularly participated reported up to three types of physical activities, together with the frequency per week and time spent per occasion on each activity. The reported type of exercise was classified as light, moderate, heavy or very heavy activity in accordance with the published energy expenditure requirements in terms of metabolic equivalents (MET) for different physical activities [21]. The time spent on recreational exercise was multiplied by the corresponding MET value (light 2, moderate 4, heavy 6 or very heavy 8) to yield a MET-hour score.

Statistical analysis. Differences in means, medians and proportions of confounding factors across the coffee and green tea consumption categories were statistically tested by one-way ANOVA, the Kruskal–Wallis test and the chi square test respectively. Analysis of covariance was used to calculate the mean concentrations of fasting and 2-h post-load plasma glucose according to the consumption of each beverage in subjects without known type 2 diabetes, with adjustment for possible confounding effects of hospital, rank in the SDF, parental history of diabetes, BMI, cigarette smoking, alcohol intake and leisure time physical activity. In addition, as coffee and green tea drinking were inversely correlated (Spearman rank correlation –0.15), we mutually adjusted for coffee and green tea consumption. Odds ratios and 95% confidence intervals of IFG, IGT, newly diagnosed type 2 diabetes and known type 2 diabetes in relation to levels of coffee and green tea consumption, were obtained from multiple logistic regression analysis while adjusting for the above-mentioned confounding variables, using normal glucose tolerance as the referent group. Trends of the association were assessed by the Wald statistic in logistic regression analysis, in which ordinal values were assigned to categories of each factor.

Categories of coffee and green tea consumption were both defined as <1, 1–2, 3–4 and ≥5 cups per day. BMI was divided into quartiles using the 25th, 50th and 75th percentiles in the distribution as cut-off points. Use of BMI as a continuous vari-

Table 1. Potential confounding variables according to daily coffee consumption

Variable	Coffee consumption (cups per day)								p value[a]
	<1		1–2		3–4		≥5		
	(n=1145)		(n=717)		(n=805)		(n=557)		
Age (years)	52.4	(0.9)	52.4	(1.0)	52.4	(0.9)	52.4	(0.9)	0.73
Examination at Fukuoka Hospital	54		62		66		73		0.001
Low ranks in the SDF	67		64		61		54		0.001
Parental history of diabetes	10		8		10		11		0.44
BMI (kg/m^2)	23.9	(2.6)	24.0	(2.7)	23.6	(2.5)	24.0	(2.7)	0.009
Ever-smoking	68		72		77		84		0.001
Cigarette-years	510	(300–640)	600	(400–720)	600	(400–750)	640	(500–900)	0.0001
Current alcohol use	86		83		82		77		0.003
Alcohol (ml/day)	48	(25–74)	46	(25–72)	45	(26–71)	44	(22–71)	0.13
MET-hours per week	16	(5–30)	16	(6–27)	14	(4–27)	13	(1–27)	0.002

Data are percentages, means (SD) or medians (interquartile ranges). [a] One-way ANOVA for mean difference, the Kruskal–Wallis test for median difference and the chi-square test for percentage difference

Table 2. Potential confounding variables according to daily green tea consumption

Variable	Green tea consumption (cups per day)								p value[a]
	<1		1–2		3–4		≥5		
	(n=453)		(n=622)		(n=1083)		(n=1066)		
Age (years)	52.4	(0.9)	52.4	(0.9)	52.4	(1.0)	52.4	(0.9)	0.68
Examination at Fukuoka Hospital	66		63		64		58		0.002
Low ranks in the SDF	65		60		62		63		0.45
Parental history of diabetes	10		10		10		9		0.83
BMI (kg/m^2)	24.1	(2.8)	23.8	(2.5)	23.8	(2.5)	23.9	(2.6)	0.25
Ever-smoking	74		75		75		72		0.31
Cigarette-years	600	(400–800)	600	(400–700)	600	(400–720)	600	(400–720)	0.27
Current alcohol use	85		86		84		79		0.02
Alcohol (ml/day)	45	(23–73)	47	(25–73)	45	(25–71)	45	(24–72)	0.78
MET-hours per week	12	(0–27)	15	(4–27)	16	(6–28)	16	(4–28)	0.12

Data are percentages, means (SD) or medians (interquartile ranges). [a] One-way ANOVA for mean difference, the Kruskal–Wallis test for median difference and the chi-square test for percentage difference

able did not change the results. Rank in the SDF was divided into two categories (low or high); cigarette smoking was divided into four categories (never-smokers and tertiles of cigarette-years in ever-smokers); alcohol intake was stratified into five categories (never-drinkers, past-drinkers and tertiles of alcohol consumed per day in current drinkers); and leisure time physical activity was divided into four levels (no regular exercise and tertiles of MET-hours per week in regular participants). Age was within a limited range from 46–59 years, and 98% of the subjects were aged 50–54 years. Age was thus not adjusted for in analysis. Repeated analyses controlling for age as a continuous variable produced essentially the same results as reported below. Indicator variables representing categories of the above-mentioned confounding factors were included in the models as independent variables.

Two-sided p values less than 0.05 were regarded as statistically significant. All analyses were performed using Statistical Analysis System (SAS), Version 6.12 (SAS Institute, Cary, N.C., USA).

Results

Among the 3224 male officials, there were 204 (6%) prevalent cases of IFG, 568 (18%) of IGT, 171 (5%) of newly diagnosed type 2 diabetes, and 187 (6%) of known type 2 diabetes.

The characteristics of the study sample varied according to the level of coffee consumption (Table 1). Men in the higher coffee consumption categories were more likely to be examinees at the Fukuoka Hospital and were more likely to have achieved a higher rank in the SDF. The frequency of ever-smokers and the cumulative amount of cigarettes smoked increased with increased coffee consumption. Furthermore, subjects with a higher intake of coffee were less likely to be current drinkers of alcohol and were less likely to participate in physical exercise during leisure time. BMI varied with coffee consumption; however, this associa-

Table 3. Plasma glucose concentrations according to daily consumption of coffee or green tea

	Daily consumption (cups per day)								*p* value for trend
	<1		1–2		3–4		≥5		
	Crude mean (SEM)								
Coffee									
Fasting (mmol/l)	5.63	(0.02)	5.52	(0.03)[a]	5.51	(0.03)[a]	5.49	(0.03)[a]	0.0003
2-h post-load (mmol/l)	6.92	(0.06)	6.74	(0.08)	6.60	(0.07)[a]	6.66	(0.09)[a]	0.001
Green tea									
Fasting (mmol/l)	5.60	(0.04)	5.57	(0.03)	5.53	(0.02)	5.55	(0.02)	0.18
2-h post-load (mmol/l)	6.76	(0.10)	6.71	(0.08)	6.68	(0.06)	6.86	(0.06)	0.23
	Adjusted mean (SEM)[b]								
Coffee									
Fasting (mmol/l)	5.60	(0.02)	5.52	(0.03)[a]	5.53	(0.03)[a]	5.52	(0.03)[a]	0.02
2-h post-load (mmol/l)	6.92	(0.06)	6.74	(0.07)	6.63	(0.07)[a]	6.63	(0.09)[a]	0.001
Green tea									
Fasting (mmol/l)	5.59	(0.04)	5.57	(0.03)	5.53	(0.02)	5.54	(0.02)	0.16
2-h post-load (mmol/l)	6.71	(0.09)	6.72	(0.08)	6.69	(0.06)	6.86	(0.06)	0.15

Men with known type 2 diabetes were excluded from the analysis. [a] $p<0.05$ vs drinking <1 cup per day; [b] adjusted for hospital, rank, parental history of diabetes, BMI, smoking, alcohol intake, leisure time physical activity, and either green tea or coffee intake

Table 4. Odds ratio for each glucose tolerance status according to level of coffee consumption

Glucose tolerance status		Coffee consumption (cups per day)				*p* value for trend
		<1	1–2	3–4	≥5	
Normal	No.	680	475	557	382	
IFG	No.	81	42	49	32	
	Crude OR	1.0 (referent)	0.7 (0.5–1.1)	0.7 (0.5–1.1)	0.7 (0.5–1.1)	0.07
	Adjusted OR[a]	1.0 (referent)	0.8 (0.5–1.2)	0.9 (0.6–1.3)	0.9 (0.6–1.4)	0.53
IGT	No.	234	138	116	80	
	Crude OR	1.0 (referent)	0.8 (0.7–1.1)	0.6 (0.5–0.8)	0.6 (0.5–0.8)	<0.0001
	Adjusted OR[a]	1.0 (referent)	0.8 (0.6–1.1)	0.6 (0.5–0.8)	0.6 (0.4–0.8)	<0.0001
Newly diagnosed type 2 diabetes	No.	70	30	40	31	
	Crude OR	1.0 (referent)	0.6 (0.4–1.0)	0.7 (0.5–1.0)	0.8 (0.5–1.2)	0.18
	Adjusted OR[a]	1.0 (referent)	0.6 (0.4–1.0)	0.8 (0.5–1.2)	0.8 (0.5–1.3)	0.37
Known type 2 diabetes	No.	80	32	43	32	
	Crude OR	1.0 (referent)	0.6 (0.4–0.9)	0.7 (0.4–1.0)	0.7 (0.5–1.1)	0.06
	Adjusted OR[a]	1.0 (referent)	0.6 (0.4–1.0)	0.7 (0.4–1.0)	0.7 (0.4–1.1)	0.09

Data are odds ratios (95% CI). [a] Adjusted for hospital, rank, parental history of diabetes, BMI, smoking, alcohol intake, leisure time physical activity and green tea intake. OR, odds ratio

tion was not simple. Few variables were associated with green tea consumption (Table 2). Subjects with a higher intake of green tea were less likely to have undergone their health examination at the Fukuoka Hospital and were less likely to be current alcohol users.

Table 3 summarises the crude and adjusted mean concentrations of fasting and 2-h post-load plasma glucose according to coffee and green tea consumption, excluding those with known type 2 diabetes. Subjects who drank coffee on a daily basis had lower fasting and post-load plasma glucose levels. Surprisingly, the reduction in post-load plasma glucose concentrations with increasing levels of coffee intake was more pronounced than the corresponding decrease in fasting plasma glucose concentrations. Compared with those who did not consume coffee on a daily basis, fasting and post-load glucose concentrations were 1.5% and 4.3% lower in men who drank 5 cups of coffee or more per day respectively. Green tea consumption was not inversely associated with either fasting or

Table 5. Odds ratio for each glucose tolerance status according to level of green tea consumption

Glucose tolerance status		Green tea consumption (cups per day)				*p* value for trend
		<1	1–2	3–4	≥5	
Normal	No.	277	414	735	668	
IFG	No.	35	48	64	57	
	Crude OR	1.0 (referent)	0.9 (0.6–1.5)	0.7 (0.4–1.1)	0.7 (0.4–1.1)	0.04
	Adjusted OR [a]	1.0 (referent)	0.9 (0.6–1.5)	0.7 (0.4–1.1)	0.6 (0.4–1.0)	0.02
IGT	No.	86	103	180	199	
	Crude OR	1.0 (referent)	0.8 (0.6–1.1)	0.8 (0.6–1.1)	1.0 (0.7–1.3)	0.85
	Adjusted OR[a]	1.0 (referent)	0.8 (0.6–1.2)	0.8 (0.6–1.1)	1.0 (0.7–1.3)	0.85
Newly diagnosed type 2 diabetes	No.	25	33	49	64	
	Crude OR	1.0 (referent)	0.9 (0.5–1.5)	0.7 (0.4–1.2)	1.1 (0.7–1.7)	0.69
	Adjusted OR[a]	1.0 (referent)	1.0 (0.6–1.7)	0.8 (0.5–1.3)	1.1 (0.7–1.9)	0.56
Known type 2 diabetes	No.	30	24	55	78	
	Crude OR	1.0 (referent)	0.5 (0.3–0.9)	0.7 (0.4–1.1)	1.1 (0.7–1.7)	0.15
	Adjusted OR[a]	1.0 (referent)	0.6 (0.3–1.0)	0.7 (0.4–1.2)	1.1 (0.7–1.7)	0.25

Data are odds ratios (95% CI). [a] Adjusted for hospital, rank, parental history of diabetes, BMI, smoking, alcohol intake, leisure time physical activity and coffee intake. OR, odds ratio

post-load plasma glucose concentrations. Those with the highest green tea intake had higher post-load plasma glucose concentrations; however, this trend was not statistically significant.

Tables 4 and 5 show the crude and adjusted odds ratios of IFG, IGT, newly diagnosed type 2 diabetes and known type 2 diabetes according to levels of coffee and green tea consumption, with normal glucose tolerance as the referent group. Coffee intake was not independently associated with IFG. Odds ratios of IGT, newly diagnosed type 2 diabetes and known type 2 diabetes were generally lower than unity among coffee drinkers, although not all of the decreases in odds ratios were statistically significant. The inverse association was particularly evident for IGT ($p<0.0001$ for trend). No clear association was observed between green tea drinking and glucose tolerance status, although green tea drinkers had a reduced odds ratio for IFG.

The adjusted odds ratios of glucose intolerance (IFG, IGT and type 2 diabetes) for categories of <1, 1–2, 3–4 and ≥5 cups of coffee per day were 1.0 (referent), 0.8 (95% CI 0.6–1.0), 0.7 (95% CI 0.6–0.9) and 0.7 (95% CI 0.5–0.9) respectively ($p=0.0001$ for trend). The corresponding values for green tea were 1.0 (referent), 0.8 (95% CI 0.6–1.1), 0.8 (95% CI 0.6–1.0) and 1.0 (95% CI 0.8–1.2) respectively ($p=0.95$ for trend).

We also performed an analysis of caffeine intake, with adjustment for hospital, rank in the SDF, parental history of diabetes, BMI, cigarette smoking, alcohol intake and leisure time physical activity. As caffeine ingestion was strongly correlated with coffee consumption (Spearman correlation coefficient 0.89), coffee and caffeine were not simultaneously included in the analysis. The adjusted mean concentrations of fasting and post-load plasma glucose according to quartile of daily caffeine intake were 5.63, 5.53, 5.55 and 5.50 mmol/l ($p=0.004$ for trend) and 6.88, 6.78, 6.75 and 6.62 mmol/l ($p=0.01$ for trend) respectively. Compared with those in the lowest quartile of daily caffeine intake, fasting and post-load plasma glucose concentrations were 2.2% and 3.8% lower in men in the highest quartile. The adjusted odds ratios of glucose intolerance according to quartile of daily caffeine consumption were 1.0 (referent), 0.8 (95% CI 0.6–1.0), 0.8 (95% CI 0.6–1.0) and 0.7 (95% CI 0.5–0.8) respectively ($p=0.0005$ for trend).

Discussion

In the present study, we have demonstrated an inverse relationship between coffee consumption and glucose intolerance, particularly IGT, by using the standard glucose tolerance test. Our study adds to increasing evidence that coffee affords protection against the development of type 2 diabetes. The present findings are consistent with those of recent prospective studies [6, 8, 9, 10] and cross-sectional studies [7, 11], but are in disagreement with the observations reported by population-based studies in the Finnish population [12] and among Pima Indians [13]. With regard to the assessment of type 2 diabetes, the majority of studies used self-reported questionnaires and/or registers of diabetic patients receiving treatment, whereas few studies adopted the standard glucose tolerance test [11, 13].

Although coffee contains many compounds which mediate a variety of physiological functions, the main biological effects of coffee drinking have been attributed to caffeine [22]. A prospective study in the