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Genetic Polymorphism in Cytochrome *P*450 7A1 and Risk of Colorectal Cancer: The Fukuoka Colorectal Cancer Study

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Abstract

Bile acids have long been implicated in the etiology of colorectal cancer, but epidemiologic evidence remains elusive. Cholesterol 7α-hydroxylase (*CYP7A1*) is the rate-limiting enzyme in the synthesis of bile acids from cholesterol in the liver, and thus may be an important determinant of bile acid production. We examined the association between the *CYP7A1 A-203C* polymorphism and colorectal cancer. The *CYP7A1 A-203C* polymorphism was determined by the PCR-RFLP method in 685 incident cases of colorectal cancer and 778 controls randomly selected from a community in the Fukuoka area, Japan. The *CC* genotype was slightly less frequent in the case group, and the adjusted odds ratio for the *CC* versus *AA* genotype was 0.88 (95% confidence interval, 0.65-1.20). In the analysis by subsite of the colorectum, a decreased risk associated with the *CYP7A1 CC* genotype was observed for proximal colon cancer, but not for either distal colon or rectal cancer. The adjusted odds ratios (95% confidence intervals) of proximal colon cancer for the *CC* genotype were 0.63 (0.36-1.10) compared with the *AA* genotype, and 0.59 (0.37-0.96) compared with the *AA* and *AC* genotypes combined. A decreased risk of proximal colon cancer in relation to the *CC* genotype of *CYP7A1 A-203C*, which probably renders less activity of the enzyme converting cholesterol to bile acids, is new evidence for the role of bile acids in colorectal carcinogenesis. (Cancer Res 2005; 65(7): 2979-82)

Introduction

Colorectal cancer is one of the most common cancers in the world, accounting for nearly 10% of all incident cases of cancer (1). Japan has experienced a rapid increase in mortality from colorectal cancer in the past 50 years (2), and is currently among the countries with the highest incidence rates worldwide (3). Bile acids have long been implicated in the etiology of colorectal cancer. Primary bile acids such as cholic and chenodeoxycholic acids are excreted in the liver, and are degraded to secondary bile acids, mainly deoxycholic and lithocholic acids, by bacteria in the intestinal lumen. Animal studies showed that secondary bile acids promoted chemically induced colorectal cancer (4, 5), and recent *in vitro* studies have identified several molecular mechanisms of deoxycholic acid promoting colorectal carcinogenesis (6, 7).

Despite these experimental observations, epidemiologic evidence remains elusive regarding the role of bile acids in colorectal carcinogenesis. Fecal levels of secondary bile acids as well as of total bile acids are higher in populations at high risk of colorectal cancer (8, 9). Several case-control studies reported higher levels of secondary bile acids in the feces or sera in patients with colorectal cancer or adenomas as compared with those without these lesions (10–13), but the findings were not replicated in other studies (14–16). A prospective study reported a suggestive increase in the risk of colorectal cancer associated with a high ratio of serum deoxycholic to cholic acids (17). Another epidemiologic evidence is the increased risk of proximal colon cancer in individuals having the gallbladder removed (18, 19). Cholecystectomy results in increased fecal excretion of secondary bile acids, probably due to increase in the bile acid pool in the enterohepatic circulation and increased degradation of primary bile acids in the gut (20, 21).

Recent studies (22, 23), but not all (24), showed that a common genetic polymorphism of cholesterol 7α-hydroxylase (*CYP7A1 A-203C*) was associated with plasma total and low-density lipoprotein cholesterol concentrations, suggesting lower activity of the enzyme in individuals with the variant C allele. *CYP7A1* is the rate-limiting enzyme in the synthesis of bile acids from cholesterol in the liver, and thus may be an important determinant of not only plasma cholesterol levels but also bile acid production. This article examined the association between the *CYP7A1 A-203C* polymorphism and colorectal cancer in order to further clarify the role of bile acids in colorectal carcinogenesis.

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

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by the ethical committees of the Faculty of Medical Sciences, Kyushu University, and of all but two of the participating hospitals. The two hospitals had no ethical committees at the time of survey. Details of the methods have been described elsewhere (25).

Participants. Cases were 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 October 2000 to December 2003. Other eligibility criteria were: age of 20 to 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, mental competence to give informed consent and to complete the interview. Of 1,053 eligible cases, a total of 840 cases (80%) participated in the interview, and 685 out of them gave an informed consent to genotyping.

Eligibility criteria for controls were the same as described for cases except for the two items, i.e., having no diagnosis of colorectal cancer and age of 20 to 74 years at the time of selection. A total of 1,500 persons were selected as control candidates by two-stage random sampling. The number of control candidates by sex and 10-year age class were determined in accordance to sex- and age-specific numbers of estimated incident cases of colorectal cancer. The first step was a random selection of 15 small areas out of 178 in total, and then ~100 persons were randomly selected in each small area using the municipal resident registry on the basis of proportions of population in the small areas by sex and 10-year age class. A letter of invitation was sent to each candidate, and at most three additional letters of invitation were mailed to nonrespondents. A total of 833 persons participated in the survey, and 778 gave an informed consent to genotyping. The net participation rate was calculated as 60% (833/1,382), after exclusion of 118 persons for the following reasons: 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), and diagnosis of colorectal cancer after the survey (n = 5).

In both cases and controls, older persons and women were less likely to give consent to genotyping, whereas there was no material difference in residence, smoking habit, and alcohol use between individuals giving consent and those who did not (Table 1).

Procedures. DNA was extracted from the buffy coat by using a commercial kit (Qiagen GmbH, Hilden, Germany). Genotyping was done by one of the authors (T. Hagiwara) using the PCR-RFLP method. The PCR was done in a reaction mixture of 10 μL containing 0.5 units of Taq and 1 μL of template DNA with a concentration of ~50 to 150 ng/μL. The *CYP7A1* genotype was determined, as described by Han et al. (24) using primers 5′-AATGT TTTTC CCAGT TCTCT TTC-3′ (sense) and 5′-AATTA GCCAT TTGTT CATTC TATTA G-3′ (antisense). After the initial denaturation at 94°C for 4 minutes, 30 cycles of PCR were done for 30 seconds at 94°C, for 30 seconds at 53°C, and for 30 seconds at 72°C, with a final extension at 72°C for 7 minutes. The PCR product of 393 bp fragment was digested with 10 units of *Bsa*I in a reaction mixture of 20 μL for 3 hours at 50°C. The digestion results in fragments of 300 and 93 bp for the *A* allele, and those of 261, 93, and 39 bp for the *C* allele. The digested PCR products were applied to electrophoresis of 3% agarose gel (NuiSieve GTG, Rockland, ME), and visualized by ethidium bromide.

The polymorphism was referred to as *A-204C* by Couture et al. (23), but the actual site of the polymorphism is located 203 bp upstream of the transcription start site according to the latest report of the sequence http://www.ncbi.nlm.nih.gov/Genomes). This was also confirmed by our sequencing of the relevant fragment.

Statistical analysis. The association of *CYP7A1* genotypes with the risk of colorectal cancer was examined in terms of odds ratio (OR) and 95% confidence intervals (CI). ORs were obtained from multiple logistic regression analysis, including indicator variables for gender, 5-year age class, and resident area (Fukuoka City or suburban area) as covariates. Statistical significance was declared if 95% CI did not include unity. All statistical analyses were done using the SAS version 8.2 (SAS Institute, Inc., Cary, NC).

Results

Proportions of the *AA*, *AC*, and *CC* genotypes in cases of colorectal cancer were 24%, 56%, and 20%, respectively (Table 2). The corresponding proportions in the control group were 25%, 51%, and 24%, respectively. The distribution in the control group was in agreement with the Hardy-Weinberg equilibrium (P = 0.59). The *CC* genotype was slightly less frequent in the case group, and the adjusted OR for the *CC* versus *AA* genotype was slightly lower than unity, with the 95% CI including unity. When the *AA* and *AC* genotypes were combined as the referent, the adjusted OR for the *CC* genotype was 0.81 (95% CI, 0.63-1.04).

The association with *CYP7A1* polymorphism was further examined for cancers of the proximal colon, distal colon, and rectum separately (Table 3). A nearly significant decrease in the OR for the *CC* versus *AA* genotype was observed for proximal colon cancer, but not for the other sites of cancer. When the *AA* and *AC* genotypes were combined as referents, the adjusted ORs of proximal colon cancer for the *CC* genotype was significantly lower than unity.

Discussion

The present study was the first that examined the relation between a functional *CYP7A1* polymorphism (*A-203C*) and

Table 1. Characteristics of cases and controls by consent to genotyping

Variable	Cases			Controls		
	With consent	Without consent	P^*	With consent	Without consent	P^*
Number	685	155		778	55	
Mean age (y)	60.2	61.9	0.03	58.6	62.9	0.004
Male (%)	62.2	48.4	0.002	63.0	45.5	0.01
Fukuoka City (%)	61.3	57.4	0.37	64.4	67.3	0.67
Ever-smoking (%)†	56.3	53.1	0.22	59.7	41.8	0.18
Alcohol use (%)†,‡	58.9	55.4	0.22	59.3	45.0	0.33

*P values (two-sided) were based on t test or χ^2 test unless otherwise specified.
†Adjusted for sex and 5-year age class by the direct method with total number of cases or controls as standard population. P values were based on the Mantel-Haenszel method.
‡Drinking alcohol at least once per week ~5 years ago.

Table 2. Adjusted ORs and 95% CI of colorectal cancer according to *CYP7A1 A-203C* polymorphisms

CYP7A1 A-203C genotype	Number (%)		Adjusted ORs (95% CI)*
	Cases (n = 685)	Controls (n = 778)	
AA	163 (24)	193 (25)	1.00 (referent)
AC	385 (56)	399 (51)	1.13 (0.88-1.46)
CC	137 (20)	186 (24)	0.88 (0.65-1.20)

*Adjusted for gender, 5-year age class, and resident area.

colorectal cancer, and showed a decreased risk of cancer of the proximal colon, but not of the distal colon and rectum, among individuals having the *CC* genotype. This genotype is probably associated with lowered capability of synthesizing bile acids (22, 23), the findings provide further evidence to the role of bile acids in colorectal carcinogenesis.

An advantage in this large-scale case-control study is that controls were derived from free-living residents in the community. It is also notable that participation rates of eligible cases and controls were fairly high. Genotyping was done in 82% of the cases and 93% of the controls who participated in the survey. It is generally considered that selection and confounding are less likely to occur in studies of genetic polymorphisms (26, 27). It is, however, possible that use of hospital controls may cause selection bias even in the gene-disease association. For instance, individuals with high blood cholesterol levels may have been included or excluded differentially in the controls if selection had occurred in patients with cholesterol-related diseases. The study subjects were an ethnically homogenous population of Japanese, and the concern over population stratification would be negligible (28).

Since the first report by Rose et al. (29), many prospective studies have observed an inverse association between serum total or low-density lipoprotein cholesterol and colon cancer (30). Although this inverse association is generally ascribed to the effect of preclinical cancer existing at the baseline (30), an increased risk of proximal colon cancer associated with low levels of serum total cholesterol persisted 10 to 20 years later in a prospective study in Hawaii (31). Furthermore, a case-control study observed lower levels of total and low-density lipoprotein cholesterol in cases of proximal colon cancer, but not of distal colon cancer, than in controls (32). These findings are congruent with decreased risk of proximal colon cancer associated with the *CYP7A1 CC* genotype.

High-fat diets are shown to increase fecal excretion of secondary bile acids as well as of total bile acids in humans (33), and to enhance chemically induced colon carcinogenesis in animals (34). Although fat intake is strongly positively correlated with colon cancer rates among countries (35), and over time in Japan (36), studies of individuals have consistently failed to find a positive association between fat intake and colon or colorectal cancer (37). The lack of an association with fat in studies of individuals may be due to a limited variation of fat intake within populations. In this regard, the present findings emphasize the usefulness of studying functional genetic polymorphisms when study populations are homogeneous with respect to exposure to environmental factors such as nutrient and food intake.

In conclusion, a large case-control study in Japan showed a decreased risk of proximal colon cancer in individuals having the *CC* genotype of *CYP7A1 A-203C*, which probably renders less activity of the enzyme converting cholesterol to bile acids. The findings add to evidence for the role of bile acids in colorectal carcinogenesis.

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Table 3. Adjusted OR and 95% CI of colorectal cancer according to *CYP7A1 A-203C* genotypes by subsite

CYP7A1 A-203C genotype	Proximal colon (n = 150)		Distal colon (n = 232)		Rectum (n = 290)	
	No.	OR (95% CI)*	No.	OR (95% CI)*	No.	OR (95% CI)*
Model 1						
AA	39	1.00 (referent)	52	1.00 (referent)	69	1.00 (referent)
AC	88	1.09 (0.71-1.65)	129	1.18 (0.81-1.70)	159	1.09 (0.78-1.53)
CC	23	0.63 (0.36-1.10)	51	1.01 (0.65-1.57)	62	0.93 (0.62-1.39)
Model 2						
AA + AC	127	1.00 (referent)	181	1.00 (referent)	228	1.00 (referent)
CC	23	0.59 (0.37-0.96)	51	0.90 (0.63-1.29)	62	0.87 (0.63-1.22)

*Adjusted for gender, 5-year age class, and resident area.

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Genetic polymorphisms of methylenetetrahydrofolate reductase and aldehyde dehydrogenase 2, alcohol use and risk of colorectal adenomas: Self-Defense Forces Health Study

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Methylenetetrahydrofolate reductase is a key enzyme in folate metabolism, which affects DNA synthesis and methylation and is possibly linked to colorectal carcinogenesis. Alcohol and acetaldehyde have an adverse effect on folate metabolism. This study investigated the relationship of functional *MTHFR C677T* and *ALDH2* polymorphisms to colorectal adenomas with reference to alcohol consumption in a case-control study of male officials in the Self-Defense Forces (SDF) who received a preretirement health examination at two SDF hospitals. The study subjects were 452 cases of colorectal adenoma and 1050 controls with no polyp who underwent total colonoscopy. Genotypes were determined by the PCR-RFLP method using genomic DNA extracted from the buffy coat. Statistical adjustment was made for age, hospital, rank in the SDF, body mass index, cigarette-years and alcohol intake. Neither *MTHFR C677T* nor *ALDH2* showed a measurable association with colorectal adenoma. While high alcohol consumption was associated with a moderately increased risk of colorectal adenoma, neither of the two polymorphisms showed a significant effect on the association between alcohol and colorectal adenoma. Individuals with the variant alleles *ALDH2*2* and *MTHFR 677T* had a decreased risk of colorectal adenomas, showing adjusted odds ratios of 0.70 (95% confidence interval 0.49–1.00) for all adenomas and 0.57 (0.34–0.95) for large adenomas (≥ 5 mm), as compared to individuals with *ALDH2*1/1* and *MTHFR 677CC* genotypes combined. The findings may be interpreted as suggesting that folate inhibits the growth of colorectal adenomas, but further confirmation is needed. (*Cancer Sci* 2005; 96: 513–518)

Folate metabolism has drawn much attention in relation to colorectal carcinogenesis.[1,2] Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme regulating folate metabolism. MTHFR irreversibly converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate.[2] The substrate of MTHFR, 5,10-methylenetetrahydrofolate, is required for conversion of deoxyuridylate to thymidylate. Insufficient thymidylate results in uracil misincorporation into DNA, leading to single-strand and double-strand breaks and increasing the incidence of DNA misrepair.[3,4] On the other hand, 5-methyltetrahydrofolate is a methyl donor in the remethylation of homocystein to methionine, which is required for DNA methylation. Imbalanced DNA methylation has been implicated in colorectal carcinogenesis.[5,6]

The *MTHFR C677T* polymorphism is a common functional polymorphism in exon 4, resulting in an alanine-to-valine substitution at codon 222.[7] The homozygous variant (*677TT*) has no more than 30% of normal enzyme activity, and heterozygotes (*677CT*) also seem to have lower activity of the enzyme.[7] Several,[8–12] but not all,[13–15] epidemiological studies have shown a decreased risk of colorectal cancer associated with the *MTHFR 677TT* genotype, especially in individuals with high folate intake and/or low alcohol intake. On the other hand, a limited number of studies addressed the relationship between the *MTHFR C677T* polymorphism and colorectal adenoma, a well-established precursor lesion of colorectal cancer.[16–20] These studies generally showed no association between the *MTHFR C677T* polymorphism and the risk of colorectal adenoma, but reported variable associations between alcohol intake and colorectal adenoma, dependent on the *MTHFR C677T* polymorphism.[16–20]

Alcohol has fairly consistently been shown to be associated with increased risk of colorectal cancer and adenoma.[21] Alcohol is known to exert adverse effects on folate metabolism by decreasing intestinal absorption and hepatic uptake, increasing renal excretion and cleaving folate.[22] It is also known that acetaldehyde, the first metabolite of ethanol, has a carcinogenic effect in animals.[23] In humans, most of the acetaldehyde is oxidized by the mitochondrial enzyme aldehyde dehydrogenase 2 (ALDH2) in the liver. The *ALDH2* gene contains the variant *ALDH2*2* allele, which results in

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an inactive enzyme by substitution of lysine for glutamine at codon 487.[24] Few studies reported that the heterozygous variant of *ALDH2*1/2* was associated with an increased risk of alcohol-related cancers and of colon cancer.[25–27] In the present study, we investigated the relationship between the *MTHFR C677T* and *ALDH2* polymorphisms and colorectal adenomas with reference to the interaction of alcohol consumption in middle-aged Japanese men. The gene–gene interaction was also a matter of interest.

Materials and Methods

Subjects

Study subjects were male officials in the Self-Defense Forces (SDF) who received a preretirement health examination at the SDF Fukuoka Hospital (Kasuga, Japan) or Kumamoto Hospital (Kumamoto, Japan) during the period January 1997 to March 2001. The preretirement health examination is a nationwide program offering a comprehensive medical examination to those retiring from the SDF. Details of the preretirement health examination have been described elsewhere.[28,29] In addition to blood samples for routine use in the health examination, a sample of 7 mL fasting venous blood was obtained for the purpose of medical research in general, not specifically for genetic study, with written informed consent. At the time of recruitment of the study subjects, there was no committee investigating ethical issues of epidemiological or genetic studies. However, the study was approved by the ethical committee of Kyushu University (Fukuoka, Japan) in the year 2002, and genotyping was carried out after individuals were made completely anonymous.

The present study included 452 cases of histologically confirmed colorectal adenoma and 1050 controls with no polyp who underwent total colonoscopy. In a consecutive series of 2459 men, five men refused to participate in the survey and a total of 319 men were excluded for not receiving colonoscopy ($n = 77$) or having a prior history of colectomy ($n = 17$), colorectal polypectomy ($n = 226$), malignant neoplasms ($n = 33$) or inflammatory bowel disease ($n = 2$). Some of the men had two or more reasons for exclusion. In the remaining 2135 men, colonoscopic findings were classified as colorectal cancer ($n = 1$), polyp ($n = 938$), non-polyp benign lesion such as diverticula ($n = 123$) and normal ($n = 1073$). The cases studied comprised 461 men out of the 938 with colorectal polyps who were found to have adenoma without *in situ* or invasive carcinoma, and the controls were 1067 men of the 1196 with normal or non-polyp benign lesions who underwent total colonoscopy. Finally, 26 men were excluded due to lack of DNA sample or unsuccessful genotyping of either polymorphism, leaving 452 cases and 1050 controls in the analysis.

Cases of adenoma with sizes of < 5, 5–9 and ≥ 10 mm (the largest size for multiple adenomas) numbered 264, 151 and 37, respectively. The analysis by subsite was confined to adenoma cases with total colonoscopy ($n = 433$). Numbers of cases having adenomas at the proximal colon alone, the distal colon and/or rectum alone, and both proximal and distal segments were 109, 218 and 106, respectively; rectal adenomas and distal colon adenomas were combined because cases with rectal adenomas alone were few ($n = 40$). Cases of large adenomas (≥ 5 mm) accounted for 37%, 32% and 63% of those having adenomas at the proximal segment only, distal segment only and both segments, respectively.

Lifestyle questionnaire

A self-administered questionnaire was used to ascertain smoking habits, alcohol consumption and other lifestyle factors prior to colonoscopy. Smokers were defined as those who had ever smoked cigarettes daily for at least 1 year. Both current and past smokers were asked about the average number of cigarettes smoked per day and total years of smoking. Cumulative exposure to cigarette smoking was expressed as cigarette-years, which were calculated by multiplying the average number of cigarettes per day by the total years of smoking. Alcohol drinkers were defined as those having drunk alcoholic beverages at least once a week for at least 1 year. Current drinkers reported the consumption of five types of alcoholic beverages (sake, shochu, beer, whisky/brandy and wine) on average in the past year, and their daily intake of ethanol was estimated. Cigarette smoking was classified into 0, 1–399, 400–799 and 800 cigarette-years, and alcohol use was categorized into never, past and current use with consumption of < 30, 30–59 or 60 mL ethanol per day. Body mass index was calculated by dividing weight in kilograms by squared height in meters, and was categorized into four levels using quartiles in the distribution in the control group. The SDF rank was classified into low, middle and high ranks.

Genotyping

DNA was extracted from the buffy coat using a commercial kit (Qiagen, Hilden, Germany). Genotyping was carried out using the PCR-RFLP method with electrophoresis on a 3% agarose gel (NuiSieve GTG) and visualization by ethidium bromide. The PCR was carried out in a reaction mixture of 10 μL containing 0.5 U *Taq* and 1 μL template DNA with a concentration of approximately 50–150 ng/μL. The *MTHFR C677T* genotype was determined, as described by Frosst *et al.*,[7] using primers 5′-TGAAG GAGAA GGTGT CTGCG GGA-3′ (sense) and 5′-AGGAC GGTGC GGTGA GAGTG-3′ (antisense). The PCR product was digested with *Hin*fI, which cleaves the 198-bp PCR product into two fragments of 175 and 23 bp when the *C677T* mutation exists.

The *ALDH2* polymorphism was determined using a method described elsewhere.[25] The primers used were 5′-CAAAT TACAG GGTCA ACTGC T-3′ (sense) and 5′-CCACA CTCAC AGTTT TCTCT T-3′ (antisense). The restriction enzyme *Ear*I digested the 135-bp PCR product into two fragments of 112 and 23 bp in the case of the wild-type allele (*ALDH2*1*).

Statistical analysis

Odds ratio (OR) and 95% confidence interval (CI) were obtained by logistic regression analysis; 95% CI was derived from the standard error for the logistic regression coefficient. Statistical adjustment was made for age (continuous variable), hospital, SDF rank, body mass index, cigarette-years and alcohol intake using indicator variables for the above-mentioned categories of the covariates. In evaluating the interaction with alcohol use, the highest two categories of alcohol intake (30–59 mL/day and 60 mL/day) were combined, and the remaining categories (lifelong non-drinking, past alcohol use and < 30 mL/day) were also combined into one group because no

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Table 1. Relationship between *MTHFR C677T* and *ALDH2* polymorphisms and colorectal adenoma

Genotype	Cases		Controls		Crude OR	
	n	%	*n*	%	Adjusted OR	(95% CI)†
MTHFR C677T						
CC	182	40.3	399	38.0	1.00	1.00 (referent)
CT	203	44.9	496	47.2	0.90	0.90 (0.71–1.16)
TT	67	14.8	155	14.8	0.95	0.93 (0.66–1.32)
ALDH2						
1/1 (wild type)	299	66.2	605	57.6	1.00	1.00 (referent)
1/2	137	30.3	390	37.1	0.71	0.81 (0.62–1.05)
2/2 (variant)	16	3.5	55	5.2	0.59	0.67 (0.35–1.27)

†Adjusted for age, hospital, rank, cigarette smoking, alcohol consumption (lifelong non-use, former use, current use of < 30, 30–59, or ≥ 60 mL alcohol/day) and body mass index. CI, confidence interval; OR, odds ratio.

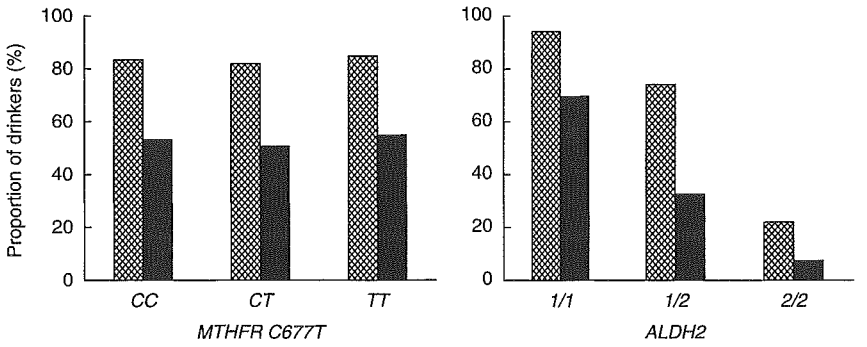

Fig. 1. Proportions (%) of current alcohol drinkers (hatched bar) and those consuming ≥ 30 mL alcohol per day (black bar) according to *MTHFR C677T* and *ALDH2* genotypes.

measurable difference in the OR was observed within each of the two combined groups (see below). When the gene–gene interaction was examined, the rare variant homozygotes of *ALDH2*2/2* were excluded, and the *MTHFR 677CT* and *677TT* genotypes were combined in some analyses to avoid potential random variation due to small numbers. Statistical assessment of the interaction was carried out on the basis of the likelihood ratio test by adding cross-product terms of indicator variables representing genotypes and alcohol drinking categories. Two-sided *P*-values less than 0.05 were regarded as statistically significant. All computations in these analyses were carried out using the Stata Statistical Software Release 8.0 (Stata Corporation, College Station, TX, USA).

Results

Among the controls, the frequencies of the *CC*, *CT* and *TT* genotypes of the *MTHFR C677T* polymorphism were 38%, 47% and 15%, respectively. The frequencies of *ALDH2*1/1*, *ALDH2*1/2* and *ALDH2*2/2* were 58%, 37% and 5%, respectively (Table 1). The distributions of the *MTHFR C677T* and *ALDH2* genotypes were each in agreement with the Hardy–Weinberg equilibrium. While alcohol use did not vary with the *MTHFR C677T* polymorphism, alcohol consumption was progressively less frequent with increasing numbers of the *ALDH2*2* allele (Fig. 1). High consumption of alcohol was associated with a moderate increase in the risk of colorectal adenoma. Adjusted OR (95% CI) of colorectal adenomas for lifelong non-drinkers, past drinkers and current drinkers consuming < 30, 30–59 or > 60 mL alcohol per day were 1.00 (referent), 1.08 (0.52–2.23), 0.90 (0.60–1.34), 1.46 (1.00–2.14) and 1.50 (1.02–2.22), respectively, when the genetic polymorphisms were not taken into account.

There was no measurable association between the *MTHFR C677T* polymorphism and colorectal adenoma (Table 1). Individuals with the *ALDH2*2* allele showed non-significant decreases in the OR of colorectal adenomas even after adjustment for alcohol consumption and other covariates.

When the relationship between the *MTHFR C677T* and *ALDH2* polymorphisms and colorectal adenoma was analyzed in combination with alcohol consumption (Table 2), the interaction with alcohol consumption was almost null for either *MTHFR C677T* or *ALDH2*. High alcohol consumption (≥ 30 mL/day) was related to a moderate increase in the risk of colorectal adenoma regardless of these genetic polymorphisms. The adjusted OR for high versus low alcohol consumption were 1.44 (95% CI 1.04–2.01) among the *ALDH2*1/1* homozygotes and 1.53 (95% CI 1.01–2.32) among the *ALDH2*1/2* heterozygotes. A separate analysis was not carried out for the *ALDH2*2/2* variant homozygotes because cases and controls with high alcohol consumption were extremely few.

The association of the combined genotype of *MTHFR C677T* and *ALDH2* with colorectal adenomas was examined after exclusion of those with the *ALDH2*2/2* genotype (Table 3). Although the interaction between the two polymorphisms was not statistically significant, individuals with the *MTHFR 677T* allele seemed to have a lowered risk of colorectal adenoma when they were *ALDH2*1/2* heterozygotes. Compared with those individuals having the *ALDH2*1/1* and *MTHFR 677CC* genotypes, individuals with the *ALDH2*1/2*

Table 2. Relationship between the *MTHFR C677T* and *ALDH2* polymorphisms and colorectal adenoma in combination with alcohol consumption

Genotype	< 30 mL alcohol/day		≥ 30 mL alcohol/day		Interaction
	n†	OR (95% CI)‡	n†	OR (95% CI)‡	
MTHFR C677T					
CC	62/186	1.00 (referent)	120/213	1.58 (1.09–2.29)	$P = 0.99$
CT	75/244	0.90 (0.61–1.34)	128/252	1.43 (0.99–2.06)	
TT	23/70	0.95 (0.54–1.67)	44/85	1.44 (0.90–2.32)	
ALDH2					
1/1 (wild type)	67/185	1.00 (referent)	232/420	1.41 (1.01–1.96)	$P = 0.90$
1/2	79/264	0.80 (0.55–1.18)	58/126	1.18 (0.77–1.82)	
2/2 (variant)	14/51	0.70 (0.36–1.37)	2/4	1.50 (0.26–8.61)	

†Numbers of cases/controls. ‡Adjusted for age, hospital, rank, cigarette smoking and body mass index. CI, confidence interval; OR, odds ratio.

Table 3. Relationship between the combined genotypes of *MTHFR C677T* and *ALDH2* and colorectal adenoma

MTHFR C677T	*ALDH2*1/1*		*ALDH2*1/2*		Interaction
	n†	OR (95% CI)‡	n†	OR (95% CI)‡	
CC	122/242	1.00 (referent)	58/132	1.02 (0.69–1.52)	$P = 0.41$
CT	132/272	0.96 (0.71–1.31)	60/200	0.69 (0.47–1.02)	
TT	45/91	0.97 (0.63–1.49)	19/58	0.72 (0.40–1.29)	
CC	122/242	1.00 (referent)	58/132	1.02 (0.69–1.52)	$P = 0.18$
CT + TT	177/363	0.96 (0.72–1.29)	79/258	0.70 (0.49–1.00)§	

†Numbers of cases/controls. ‡Adjusted for age, hospital, rank, cigarette smoking, alcohol consumption (< 30 or ≥ 30 mL/day) and body mass index. §$P = 0.048$. CI, confidence interval; OR, odds ratio.

Table 4. Relationship between the combined genotypes of *MTHFR C677T* and *ALDH2* and colorectal adenoma according to size and site of adenoma

MTHFR C677T	*ALDH2*1/1*		*ALDH2*1/2*		Interaction
	n†	OR (95% CI)‡	n†	OR (95% CI)‡	
Small adenomas					
CC	65/242	1.00 (referent)	35/132	1.12 (0.69–1.81)	$P = 0.25$
CT + TT	104/363	1.04 (0.73–1.48)	50/258	0.81 (0.53–1.25)	
Large adenomas					
CC	57/242	1.00 (referent)	23/132	0.91 (0.52–1.58)	$P = 0.36$
CT + TT	73/363	0.87 (0.59–1.29)	29/258	0.57 (0.34–0.95)	
Proximal colon adenomas					
CC	28/242	1.00 (referent)	12/132	0.92 (0.44–1.93)	$P = 0.98$
CT + TT	42/363	1.04 (0.62–1.73)	25/258	0.97 (0.53–1.77)	
Distal colon and rectal adenomas					
CC	58/242	1.00 (referent)	30/132	1.13 (0.68–1.89)	$P = 0.30$
CT + TT	83/363	0.96 (0.65–1.40)	38/258	0.77 (0.48–1.23)	

†Numbers of cases/controls. ‡Adjusted for age, hospital, rank, cigarette smoking, alcohol consumption (< 30 or ≥ 30 mL/day) and body mass index. CI, confidence interval; OR, odds ratio.

genotype and *MTHFR 677T* allele (*677CT* and *677TT* combined) had a statistically significant decrease in the risk of adenomas. There was no interaction between the combined genotype and alcohol consumption (data not shown). With allowance for the combined genotypes of the *MTHFR C677T* and *ALDH2* polymorphism, adjusted OR for high (≥ 30 mL/day) versus low (< 30 mL/day) consumption of alcohol was 1.43 (95% CI 1.10–1.85).

A decreased risk of colorectal adenoma associated with the *MTHFR 677T* allele among *ALDH2*1/2* heterozygotes was more evident for large adenomas than for small adenomas, and slightly so for distal adenomas than for proximal adenomas (Table 4). The decrease in the OR of large adenomas was statistically significant. Again, the combined genotype of *MTHFR C677T* and *ALDH2* did not modify the association with alcohol consumption for either small or large adenomas and for either proximal or distal adenomas (data not shown). The adjusted OR (95% CI) for high versus low alcohol consumption with further adjustment for the combined genotype were: small adenomas, 1.44 (1.05–1.96); large adenomas, 1.45 (1.00–2.10); proximal adenomas, 1.31 (0.84–2.06); and distal adenomas, 1.72 (1.22–2.44).

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Discussion

The present study showed no measurable association between either the *MTHFR C677T* or *ALDH2* polymorphisms and colorectal adenoma. Neither of the polymorphisms showed an effect on the association between alcohol consumption and colorectal adenomas.

Previous studies have generally found no measurable association between the *MTHFR C677T* polymorphism and colorectal adenoma.[16–20] Results from these studies, however, are disparate regarding the interaction between the *MTHFR C677T* polymorphism and alcohol consumption on the risk of colorectal adenoma. Of the five studies,[16–20] two showed an increased risk of colorectal adenoma associated with high alcohol intake among those with the *677TT* genotype, while no clear association with alcohol was seen among those with the *677CC* or *CT* genotypes.[18,20] An earlier study of officials in the SDF also observed an increased risk among the *677TT* homozygotes with high alcohol consumption.[19] On the contrary, the Minnesota case-control study reported a significant positive association with alcohol intake in those with the *677CC* genotype (trend $P = 0.005$) and an inverse association in those with the *677TT* genotype (trend $P = 0.10$), showing a statistically significant interaction.[17] In the Nurses' Health Study in the United States,[16] a significant increase in risk was observed among the *677TT* homozygotes with low alcohol consumption. Some of the inconsistent findings as to the interaction between alcohol intake and the *MTHFR C677T* polymorphism may be due to chance. Alternatively, inconsistency among the studies may be related to different folate levels in the study populations. The *MTHFR C677T* polymorphism could exert opposite effects in colorectal carcinogenesis depending on the folate pool because thymidylate synthesis and DNA methylation proceed at the expense of each other.

The present study did not examine the association between risk of colorectal adenoma and the *MTHFR A1298C* polymorphism, another functional polymorphism in the *MTHFR* gene.[2] This polymorphism seems less relevant to the risk of colorectal cancer or adenomas,[11,12,20] although a decreased risk of colon cancer was reported inconsistently for the *1298CC* genotype in a subgroup analysis.[13] The *A1298C* polymorphism was virtually unrelated to colorectal cancer in a large case-control study in Japan.[12]

The *ALDH2*1/2* heterozygosity was shown to be associated with an increased risk of alcohol-related cancers and of colon cancer among Japanese alcoholics.[25] A case-control study in Japan also showed a slightly greater increase in the risk of colon cancer, but not rectal cancer, associated with high alcohol consumption among *ALDH2* heterozygtes compared with *ALDH2* wild-type homozygotes.[26] These results, taken together with the carcinogenic property of acetaldehyde, suggest that the *ALDH2* variant allele might confer a greater risk of colorectal adenoma associated with alcohol consumption. The present study, however, provided no evidence for an interaction between the *ALDH2* polymorphism and alcohol use on the risk of colorectal adenoma, whereas alcohol use itself was associated with a moderately increased risk of colorectal adenoma. The lack of interaction between alcohol and the *ALDH2* polymorphism indicates that acetaldehyde metabolism in the liver may not be linked with the development of colorectal adenoma or cancer. Bacterial production of acetaldehyde in the colon is an alternative mechanism by which alcohol may enhance colorectal carcinogenesis.[30] In rats, ethanol consumption results in a substantial increase in the concentration of acetaldehyde and decreases folate levels in the colonic mucosa.[31] Furthermore, incubation of human colonic contents with alcohol results in significant production of acetaldehyde.[32] It is notable that an increased risk of colorectal adenoma and cancer associated with alcohol consumption have been observed fairly consistently in different populations, including Caucasians[21] in whom the frequency of the *ALDH2*2* allele is virtually zero.[24]

Interestingly, the combination of variant alleles (*ALDH2*2* and *MTHFR 677T*) was associated with a decreased risk of colorectal adenoma, especially of large adenomas. The findings suggest that individuals with the *MTHFR 677T* allele may have a lower risk of colorectal adenoma under certain conditions. It is possible that individuals with the *ALDH2*2* allele may have had favorable dietary patterns other than lower consumption of alcohol, especially in terms of folate intake. The seemingly greater decrease in the risk of large adenomas associated with the coexistence of the *ALDH2*2* and *MTHFR C677T* alleles may be ascribed to chance because cases were much fewer in the subgroup analysis by size. Nonetheless, this finding is compatible with a decreased risk of colorectal cancer associated with the *MTHFR 677TT* genotype under high-folate and low-alcohol conditions, and may be interpreted as suggesting that folate inhibits the growth of colorectal adenoma.

Different molecular alterations have been implicated in carcinogenesis of the proximal and distal sites of the colorectum.[33,34] The *MTHFR 677TT* genotype was more strongly[10] or exclusively[14] associated with decreased risk of proximal colon cancer. In the present study, however, there was no clear difference in the association with either the *MTHFR C677T* or *ALDH2* polymorphism between proximal and distal adenomas, although an increased risk of adenomas associated with high alcohol consumption seemed to be slightly greater for distal adenomas.

The present study had methodological advantages in that colonoscopy was done almost unselectively in a defined population and the absence of polyp lesions was confirmed in the control subjects by total colonoscopy. The study subjects were not representative of Japanese men in the general population, but selection was unlikely to exist with regard to the genetic polymorphisms under study. The allele frequency of *MTHFR 677T* (38% in the controls) is quite similar to those reported in random or non-random samples of Japanese populations elsewhere.[12,35,36] The frequency of the *ALDH2*2* allele (24% in the controls) did not differ greatly from the somewhat variable frequencies observed in Japan; for example, the variant allele accounted for 17% in a random sample of 324 adult residents[35] and 28% among 241 non-cancer outpatients at a hospital.[36]

The lack of information regarding folate intake was a weakness of the present study. Although the questionnaire included questions on consumption frequency of selected food items, the method of estimating folate intake has not been established in the Self-Defense Forces Health Study. Such information would be of value in clarifying the role of

the *MTHFR C677T* polymorphism in the occurrence of colorectal adenoma. Whereas the statistical power was fairly high in addressing the overall association with each polymorphism, smaller numbers in the analysis of interaction necessarily resulted in a substantial decrease in the power. For example, the power of detecting a 1.5-fold increase in risk for the *ALDH2*1/2* versus *ALDH2*1/1* genotype at the 5% significance level (two-sided) was roughly estimated to be 93%, but it was no more than 44% when such an increase in risk was sought in individuals with *ALDH2*1/2* and high alcohol use compared with those with *ALDH2*1/1* and low alcohol use.

In summary, a case-control study of Japanese men showed no measurable association of either the *MTHFR C677T* or *ALDH2* polymorphisms with colorectal adenoma. While alcohol intake was associated with a moderate increase in the risk of colorectal adenoma, neither of the two polymorphisms modified the relationship between alcohol consumption and colorectal adenoma. The combination of *ALDH2*2* and *MTHFR 677T* alleles was associated with a decreased risk of colorectal adenoma, especially of large adenomas, but the findings need further confirmation.

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Original Article

Class of Antihypertensive Drugs, Blood Pressure Status, and Risk of Cardiovascular Disease in Hypertensive Patients: A Case-Control Study in Japan

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The purpose of this study was to compare the effect of different classes of antihypertensives on the risk of cardiovascular events in a case-control study of hypertensive patients. The subjects consisted of 171 hypertensive patients who had experienced a cardiovascular event and 537 randomly selected hypertensive controls who were matched to the cases by gender, age, and hospital/clinic. Both cases and controls had been under antihypertensive medication for at least 6 months before the onset of the cardiovascular event (cases) or before the enrollment (controls). A total of 134 physicians across the nation recruited cases and controls, and reported details of the prescription of antihypertensives and clinical and behavioral variables of their patients. Although there was no measurable difference in the risk of cardiovascular events according to the class of antihypertensives, statistically significant increases in the risk of cardiovascular events were observed for non-use of calcium antagonists among patients with angina pectoris and for non-use of the renin-angiotensin system inhibitor (angiotensin-converting enzyme inhibitor and angiotensin II receptor blockers combined) among patients with diabetes mellitus. Higher levels of blood pressure were associated with an increased risk of cardiovascular events. The findings suggest that appropriate control of blood pressure is more important in the treatment of hypertension than the choice of antihypertensives. (*Hypertens Res* 2005; 28: 811–817)

***Key Words*: angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, calcium channel blockers, cardiovascular disease, case-control study**

Introduction

Hypertension is associated with increased risk of cardiovascular diseases, and it has been well established that pharmacological treatment for hypertension substantially reduces the risk of stroke, coronary heart disease, and other cardiovascular diseases (*1–3*). Much interest has recently been drawn to the question of whether any one class of antihypertensive drugs is more effective than the others. A meta-analysis suggested that calcium antagonists were inferior to other types of antihypertensives in reducing the risk of major cardiovascular events (*4*). In another meta-analysis, however, there was no overall difference in major cardiovascular events among the three treatment regimens of angiotensin-converting enzyme (ACE) inhibitors, calcium antagonists, and diuretics and β-

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blockers combined (*5*). The Antihypertensive and Lipid-Lowering Treatment Trial indicated that diuretic was superior to calcium antagonists and ACE inhibitors in preventing one or more major cardiovascular events (*6*, *7*). Furthermore, losartan, an angiotensin II receptor blocker, was shown to have a greater effect of reducing cardiovascular events than atenolol in a randomized trial (*8*), while a recent trial showed no overall difference in cardiovascular events between a calcium antagonist and an angiotensin II receptor blocker (*9*).

In Japan, one study reported a more frequent occurrence of cerebrovascular events in patients allocated to receive a calcium antagonist as compared with those receiving an ACE inhibitor (*10*), but another trial found no difference in cardiac events between an ACE inhibitor and a calcium antagonist (*11*). We therefore carried out a case-control study of patients under antihypertensive medication to address the question of whether the effect of antihypertensive drugs on the risk of cardiovascular events (stroke, myocardial infarction, and sudden and unexpected death) differs by the class of antihypertensive drugs.

Methods

Both cases and controls were Japanese patients who had been receiving medication for hypertension for a period of at least 6 months before the onset of the cardiovascular event (cases) or before the enrollment (controls). The cardiovascular events under study were stroke (cerebral infarction, cerebral hemorrhage, and subarachnoid hemorrhage), myocardial infarction, and sudden and unexpected death. Both cases and controls had no prior history of symptomatic stroke, myocardial infarction, or intervention procedures for the coronary, carotid, and cerebral arteries. Patients with severe life-limiting conditions such as renal dialysis and malignant neoplasm under treatment were not eligible. A total of 134 physicians across the nation participated in the study, and they were in charge of recruitment of patients and collection of information on clinical and behavioral variables. The study was designed and implemented in accordance with the Helsinki Declaration, and was approved by the Institutional Review Board of each participating institution. Written informed consent was given by each participating patient or a proxy family member if the patient was deceased at the time of recruitment.

Cases

Cases were hypertensive patients aged 50–79 years who first experienced the above-specified cardiovascular event during the period from October 2001 to March 2002. They had to have been under medication for hypertension over 6 months or longer prior to the onset of the cardiovascular event. The diagnosis of cerebral hemorrhage, cerebral infarction, and subarachnoid hemorrhage was based on acute neurological symptoms and imaging techniques such as computed tomography and magnetic resonance imaging. Myocardial infarction was defined based on clinical symptoms accompanied with diagnostic serum enzyme elevations or electrocardiographic findings. Sudden and unexpected death was defined as death occurring within 24 h after the onset of severe symptoms in the absence of known conditions other than coronary heart disease and stroke. The definition of sudden and unexpected death was a modified version of the criteria used in the Lipid Research Clinics Coronary Primary Prevention Trials (*12*).

In the consecutive series of 235 cases, 209 patients agreed to participate in the study, but 32 cases were found to be not eligible after the enrollment, and 5 cases had no matched control. Further, one case was excluded due to lack of compliance with the prescribed drug regimen. Thus 171 cases remained in the analysis. The types of cardiovascular events were as follows: cerebral infarction ($n=92$), cerebral hemorrhage ($n=18$), subarachnoid hemorrhage ($n=6$), myocardial infarction ($n=47$), and sudden and unexpected death ($n=8$).

Controls

The eligibility criteria of controls included no prior history of the cardiovascular events under study, an age of 50–79 years at the time of enrollment, and medication for hypertension for 6 months or longer before the enrollment. At the time of enrollment of each case, approximately 20 patients under medication for hypertension in a consecutive series were temporarily enrolled from the same institution as each case. Of these control candidates, at most 4 patients whose sex and age (within 5 years) were the same as those of the case were randomly selected. A total of 3,939 patients were temporarily enrolled in this manner, and 599 patients were randomly selected. Of these, 43 patients refused to participate in the study, 18 were found to be ineligible after collection of relevant information, and one had hardly taken the prescribed drug. After exclusion of these patients, 537 controls remained in the analysis. The numbers of controls matched to cases of cerebral infarction, cerebral hemorrhage, subarachnoid hemorrhage, myocardial infarction, and sudden and unexpected death were 283, 61, 23, 147, and 23, respectively.

Clinical and Behavioral Variables

The names of antihypertensive drugs and the duration of prescription before the onset of cardiovascular event (cases) or before the enrollment (controls) were reported by the participating physicians. In each case, a class of antihypertensive drugs was regarded as having been prescribed if the patient had taken it for at least 90 consecutive days at some point in the past 6 months. The study physicians also reported their judgment of the patient's compliance with the prescription (categorized as "taken 5+ days per week," "half-taken," and "hardly taken"), the frequency of clinic visits (open-ended question), and the monthly measurements of blood pressure in the period of 1–6 months prior to the event (cases) or

Table 1. Blood Pressure Status, Type of Prescribed Antihypertensives, and Other Characteristics among Cases of Cardiovascular Events and Controls

Characteristics	Cases (n=171)	Controls (n=537)
Male (%)	60.2	55.5
Age (years)[a]	68.5±7.0	68.7±6.4
Blood pressure (%)[b]		
Normal	36.3	49.3
Mild hypertension	52.0	43.9
Moderate to severe hypertension	11.7	6.7
Type of antihypertensives (%)		
Diuretics	6.4	5.4
β-Blockers	19.3	14.9
Calcium antagonists	71.3	71.1
ACE inhibitors	30.4	34.1
ARBs	23.4	22.2
Others	14.0	10.8
Good compliance (%)[c]	90.6	97.8
No. of visits (median (interquartile range))	7 (6–12)	7 (6–12)
Angina pectoris (%)	5.8	4.5
Diabetes mellitus (%)	28.7	16.6
Hypercholesterolemia (%)[d]	36.3	43.4
Nephritis/nephrosis (%)	3.5	2.4
Overweight (%)[e]	33.3	36.1
Regular physical activity (%)[f]	42.7	51.4
Current smoking (%)[f]	29.8	21.2
Current alcohol use (%)[f]	39.8	43.4

ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker. [a]Data are mean±SD. [b]Based on the classification by the Japanese Society of Hypertension (*3*). [c]Taking 5 days or more per week. [d]Serum total cholesterol ≥220 mg/dl and/or statin use. [e]Body mass index ≥25 kg/m^2. Data were missing for 12 cases and 13 controls. [f]Data were missing for 5 controls, and they were categorized as non-participants in regular physical activity, nonsmokers, or nondrinkers

enrollment (controls). The average systolic and diastolic blood pressure were obtained from the monthly records. The median number of blood pressure measurements taken was 4 in both cases and controls, and 17 cases and 26 controls had only one recorded measurement of blood pressure. Based on the average systolic and diastolic blood pressure, blood pressure status was classified as normal (including optimal and high normal), mildly hypertensive, moderately hypertensive, and severely hypertensive according to the definition of the Japan Society of Hypertension (*3*), which is identical to the WHO/ISH statement definition (*1*). Moderate and severe hypertension were combined in the analysis because patients in these two categories were few.

The physician's diagnoses of angina pectoris, diabetes mellitus, and nephritis or nephrosis were recorded, along with any use of nitrites, oral hypoglycemic drugs, insulin, and statins, and the results of serum total cholesterol measurement. Patients were defined as hypercholesterolemic if their serum total cholesterol was ≥220 mg/dl and/or if they were under statin treatment. The activity of daily living (ADL) was assessed by using 4 precoded answers: completely independent, independent but with supportive devices and aids, under partial care, and bedridden. There was no bedridden patient in either the cases or controls as defined in the eligibility criteria.

Height, body weight, physical activity, smoking habit, and alcohol use were based on the report by patients (or a proxy of the family when a patient had deceased). The answer to the question regarding physical activity was dichotomous: hardly done (<1 times per week) or regularly done (≥1 times per week). Ever-smoking was defined as the daily use of cigarettes for 1 year or longer, and a smoking habit was categorized as lifelong nonsmoking, former smoking, and current smoking. With alcohol use defined as drinking alcohol at least once per week over 1 year or longer, individuals were classified as lifelong nondrinkers, former drinkers, and current drinkers.

Statistical Analysis

Conditional logistic regression analysis was used to estimate the odds ratio (OR) and its 95% confidence interval (CI) with and without adjustment for the clinical and behavioral variables. The 95% CI was estimated by using the standard error of the conditional logistic regression coefficient. Dichotomous variables were used for compliance with antihypertensive medication, angina pectoris, diabetes mellitus, hypercholesterolemia, regular physical activity, and the ADL. Indicator variables were created to represent the three categories of blood pressure status, smoking, and alcohol use. The OR was considered to be statistically significant if the 95% CI did not include unity. Interactions between the type of antihypertensives and clinical risk factors were explored with a statistical significance level of 0.10 (two-sided). Statistical assessment of the interaction was done by the likelihood ratio test comparing the two models with and without an interaction term. All computations were made using the statistical software SAS release 8.2 (SAS Institute Inc., Cary, USA).

Results

The characteristics of cases and controls are summarized in Table 1. The age distribution in the cases and controls was almost identical, although the proportion of men was slightly greater in the case group. While there was no material difference in the class of antihypertensives between cases and controls, patients with mild and moderate/severe hypertension and those with poor compliance were more frequent in cases than in controls. Diabetes mellitus and smoking were more prevalent, and regular physical activity and alcohol use were slightly less prevalent in the cases.

As expected from the proportions for each class of antihy-

Table 2. Use of Specific Antihypertensive Drugs and the Risk of Cardiovascular Events

Type of antihypertensives	No.[a]	Crude OR[b] (95% CI)	*p*-value	Adjusted OR[c] (95% CI)	*p*-value
Diuretics	11/29	1.11 (0.52–2.34)	0.79	1.05 (0.46–2.41)	0.90
β-Blockers	33/80	1.42 (0.88–2.27)	0.15	1.44 (0.84–2.48)	0.19
Calcium antagonists	122/382	1.01 (0.68–1.50)	0.95	1.01 (0.65–1.56)	0.98
ACE inhibitors	52/183	0.82 (0.55–1.21)	0.31	0.80 (0.51–1.23)	0.31
ARBs	40/119	1.05 (0.69–1.61)	0.81	1.02 (0.63–1.66)	0.92
Others	24/58	1.35 (0.79–2.31)	0.27	1.19 (0.65–2.16)	0.57

OR, odds ratio; CI, confidence interval; ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker. [a]Numbers of cases/controls. [b]Matched with sex, age, and institution. The referent group used antihypertensive drugs other than the specified class of drugs. [c]Adjusted for blood pressure status, compliance with antihypertensives, angina pectoris, diabetes mellitus, hypercholesterolemia, regular physical activity, smoking, alcohol use, and activity of daily living in addition to the matched variables (sex, age, and hospital). The referent group used antihypertensive drugs other than the specified class of drugs.

Table 3. Risk of Cardiovascular Events for Use of Calcium Antagonists and Renin-Angiotensin System (RAS) Inhibitors Compared with Use of Other Antihypertensive Drugs

Type of antihypertensives	No.[a]	Crude OR[b] (95% CI)	*p*-value	Adjusted OR[c] (95% CI)	*p*-value
Calcium antagonists only	70/222	0.86 (0.41–1.81)	0.69	0.84 (0.37–1.90)	0.68
RAS inhibitors only[d]	38/123	0.82 (0.37–1.82)	0.63	0.78 (0.33–1.85)	0.58
Both calcium antagonists and RAS inhibitors	52/160	0.89 (0.42–1.89)	0.76	0.82 (0.36–1.90)	0.65
Others[e]	11/32	1.00 (referent)	—	1.00 (referent)	—

OR, odds ratio; CI, confidence interval. [a]Numbers of cases/controls. [b]Matched with sex, age, and institution. The referent group used antihypertensive drugs other than the specified class of drugs. [c]Adjusted for blood pressure status, compliance with antihypertensives, angina pectoris, diabetes mellitus, hypercholesterolemia, regular physical activity, smoking, alcohol use, and activity of daily living in addition to the matched variables (sex, age, and hospital). [d]RAS inhibitors included angitotensin-converting enzyme inhibitors and angiotensin II receptor blockers. [e]Drugs other than calcium antagonists and RAS inhibitors.

pertensives shown in Table 1, the crude ORs for the use of an individual class of antihypertensive drugs were not very different from unity, and the results did not change after adjustment for blood pressure status, compliance, and other cardiovascular risk factors (Table 2). On the other hand, the grade of blood pressure status was positively associated with the risk of cardiovascular events. The crude ORs (95% CIs) for normal blood pressure, mild hypertension, and moderate/severe hypertension were 1.00 (referent), 1.70 (1.14–2.53), and 2.51 (1.29–4.87), respectively. After adjustment for the covariates except compliance, the ORs (95% CIs) for normal blood pressure, mild hypertension, and moderate/severe hypertension were 1.00 (referent), 1.69 (1.10–2.60), and 2.20 (1.08–4.45), respectively.

Further analysis was done by breaking down the antihypertensive drugs into three groups: calcium antagonists; renin-angiotensin system (RAS) inhibitors, which included both ACE inhibitors and angiotensin II receptor blockers; and other drugs, including diuretics and β-blockers. The number of patients who were prescribed the combination of a calcium antagonist plus a RAS inhibitor was 52 cases and 160 controls. Very few patients received calcium antagonists and/or RAS inhibitors in combination with antihypertensives other than these drugs (data not shown). Neither calcium antagonists nor RAS inhibitors were specifically associated with the risk of cardiovascular events (Table 3).

We explored the interactions of each group of antihypertensive drugs (calcium antagonists, RAS inhibitors, and the traditional drugs) with the clinical risk factors for cardiovascular events. Possible interactions were noted between calcium antagonists and angina pectoris (interaction $p=0.037$) and between diabetes mellitus and RAS inhibitors (interaction $p=0.098$). A statistically significant 5-fold increase in the risk of cardiovascular events was observed for non-use of calcium antagonists in the presence of angina pectoris (Table 4), and non-use of RAS inhibitors was associated with a statistically significant 3-fold increase in the risk among patients with diabetes mellitus (Table 5).

Discussion

The present study did not show any material difference in the risk of cardiovascular events among hypertensive patients under medication with different types of antihypertensive

Table 4. Risk of Cardiovascular Events in Relation to the Combination of Angina Pectoris and Calcium Antagonists[a]

Angina pectoris	Calcium antagonists	No.[b]	Adjusted OR (95% CI)[c]	*p*-value
(−)	(−)	44/151	1.00 (referent)	—
(−)	(+)	117/362	1.11 (0.70–1.75)	0.65
(+)	(−)	5/4	5.08 (1.08–23.9)	0.04
(+)	(+)	5/20	0.71 (0.21–2.45)	0.59

OR, odds ratio; CI, confidence interval. [a]Interaction $p=0.037$. [b]Numbers of cases/controls. [c]Adjusted for blood pressure status, compliance with antihypertensives, diabetes mellitus, hypercholesterolemia, regular physical activity, smoking, alcohol use, and activity of daily living in addition to the matched variables (sex, age, and hospital).

Table 5. Risk of Cardiovascular Events in Relation to the Combination of Diabetes Mellitus and Renin-Angiotensin System (RAS) Inhibitors[a]

Diabetes mellitus	RAS inhibitors	No.[b]	Adjusted OR (95% CI)[c]	*p*-value
(−)	(−)	55/218	1.00 (referent)	—
(−)	(+)	67/230	1.14 (0.72–1.82)	0.57
(+)	(−)	26/36	3.01 (1.48–6.10)	0.002
(+)	(+)	23/53	1.57 (0.78–3.14)	0.20

OR, odds ratio; CI, confidence interval. [a]Interaction $p=0.098$. [b]Numbers of cases/controls. [c]Adjusted for blood pressure status, compliance with antihypertensives, angina pectoris, hypercholesterolemia, regular physical activity, smoking, alcohol use, and activity of daily living in addition to the matched variables (sex, age, and hospital).

drugs. On the other hand, there was a positive relationship between blood pressure status and the risk of cardiovascular events. The findings reiterate the importance of achieving and maintaining an appropriate level of blood pressure in the management of hypertension, *i.e.*, below 130 mmHg in systolic blood pressure and below 85 mmHg in diastolic blood pressure (*3*).

We observed that the risk of cardiovascular events increased when the RAS inhibitor was not used among the patients with diabetes mellitus and when calcium antagonists were not used in patients with angina pectoris. Because these findings were the results of an exploratory analysis seeking for possible interactions, caution should be exercised in their interpretation. Nonetheless, these findings are consistent with previous observations as regards RAS blockers and calcium antagonists.

Both ACE inhibitors and angiotensin II receptor blockers were shown to improve insulin resistance in humans as well as in animals (*13*, *14*). These RAS inhibitors also reduced the incidence of new onset diabetes mellitus (*7*, *15*). In a randomized placebo-controlled trial of hypertensive patients with diabetes mellitus (*16*), the investigators noted that a reduction in the risk of cardiovascular events associated with ACE-inhibitor treatment was greater than that attributable to the decrease in blood pressure. Furthermore, randomized trials comparing the effects of ACE inhibitors and calcium antagonists among people with hypertension and diabetes mellitus reported that the ACE inhibitors were more effective at reducing cardiovascular events (*17*, *18*), although such a differential effect as regards diabetes mellitus was not always observed (*19*). Hypertension is the most common component of metabolic syndrome (*20*), and the choice of antihypertensive drugs may be an important matter in the treatment of hypertension among patients with diabetes mellitus (*21*).

As regards calcium antagonists, randomized trials based on coronary angiography showed that calcium antagonists suppressed the occurrence of new atherosclerotic lesions and progression of minimal coronary lesions in patients with coronary artery disease, although the drugs did not affect the progression or regression of advanced atherosclerotic lesions (*22*, *23*). Amlodipine (calcium antagonist) was shown to have the effect of slowing the progression of carotid artery atherosclerosis, but not of coronary atherosclerosis (*24*), and the occurrence of myocardial infarction among patients with hypertension treated with amlodipine was less than that in those treated with an angiotensin II antagonist (*9*).

Our present findings on the type of antihypertensive drugs used in clinical practice were also of interest. In the control group, over 70% of hypertensive patients received calcium antagonists, and the ACE inhibitor was the second most common agent (34%), followed by angiotensin II receptor blockers (22%), β-blockers (15%), and diuretics (6%). These figures probably represented the use of antihypertensive drugs in Japan (*25*), since physicians across the nation participated in the study. In a survey conducted during the period from September 2000 to March 2001 in Tottori, Japan (*26*), the overall prescription rates of calcium antagonists, ACE inhibitors, angiotensin II receptor blockers, β-blockers, and diuretics were 73%, 31%, 19%, 16%, and 10%, respectively.

Selection and information bias are methodological con-

cerns in case-control studies. In the present study, the cases were patients with defined cardiovascular events in a consecutive series, and the controls were a random sample out of a consecutive series of hypertensive patients under medication. These procedures in the recruitment of cases and controls minimized selection bias and ensured comparability between cases and controls. Prescription of antihypertensive drugs, blood pressure, and cardiovascular risk factors were ascertained retrospectively, but on the basis of recorded data. In this regard, the present findings are unlikely to have been ascribable to information bias, although comorbid conditions such as hypercholesterolemia and angina pectoris may not have been ascertained accurately. Classification of the antihypertensive drugs prescribed for each patient was based on the continuous prescription for at least 90 days in the past 6 months. Prescription in the immediate past might be more relevant to the risk of cardiovascular events.

The randomized controlled trial is a preferred method for evaluating the efficacy of therapeutic treatments, but observational studies complement the evidence from randomized clinical trials. The efficacy shown in a limited group of patients may not be directly applicable in routine practice. The effectiveness of the treatments under actual clinical conditions can be addressed in observational studies (*27*, *28*). Although prospective studies are generally regarded as superior to case-control studies, results from case-control studies are as valid as those from prospective studies if prescription is determined on the basis of recorded data. As illustrated in a study showing that poor control of blood pressure was clearly associated with an increased risk of stroke in routine practice (*29*), case-control studies are a valuable method of evaluating the effectiveness of a therapeutic regimen in a society in which randomized controlled trials are difficult to implement.

Appendix

The following physicians participated in the study: [Hokkaido district] Hirotsugu Imamura, Itaru Maeda, Shin-ichiro Satoh, Fumio Ishizaka; [Tohoku district] Yoshihisa Akino, Masatoshi Onoda, Takashi Kimura, Yoshiko Shibata, Toru Chiba, Koji Tsukuda, Ryo Ito, Masato Hayashi, Etsuko Fushimi, Hirohisa Sudo, Yukio Kubota, Yoshiharu Haga; [Kanto district] Kiyohiro Akada, Nobuyoshi Hatano, Atsuro Kato, Hiroyuki Kuroki, Manabu Narimiya, Soichiro Ishimoto, Yasuaki Ishimaru, Akiyoshi Ohtsuka, Ichiro Michishita, Mitsuo Amano, Terukuni Ideura, Masao Ishii, Masataka Shoda, Masayuki Nakao, Masahiko Kanna, Toshihiko Saito, Yukihiko Miura, Susumu Takano, Seinosuke Ryu, Mitsuhiro Miyazaki, Shoji Ohba, Yoshiaki Sohara, Osamu Sakayori, Akira Yamazaki, Takashi Funada, Haruo Iwakura, Takeshi Takei, Yuji Shimizu; [Chubu district] Makihiko Saeki, Tetsuji Kosaki, Mutsuo Kusunose, Atsushi Nomura, Tomoko Katoh, Tatsuji Furuta, Taisei Kawamura, Takeshi Kondo, Shinya Hiramitsu, Masayoshi Sarai, Masato Watarai, Motohiko Nishida, Yoshihiro Hattori, Makoto Hayakawa, Kei Iida, Kohzo Kawai, Sumio Mizuno, Jun-ichi Hirai, Kouji Maeno; [Kinki district] Kazumi Matsuda, Hirofumi Kusaka, Hidefumi Ito, Hisashi Ito, Masumi Sano, Toshihiko Sano, Yo Nagahama, Naoto Minamitani, Mitsushige Ohta, Hisahiro Yu, Shigefumi Nakamura, Atsushi Moriguchi, Takaya Hasegawa, Hideo Matsui, Terutaka Tsuda, Toru Miyajima, Nobuhiko Miki, Kunio Hashimoto, Yoshiaki Fukuoka, Kazuhiro Uragami, Tetsuro Ichida, Keiko Kano, Yoko Taniguchi, Tatsuhito Nakae, Akitsugu Nishiyama, Hirofumi Takashima, Yasuo Takayama, Takayuki Nakatsuka, Masahiro Amenomori, Yuusaku Minami; [Chugoku district] Kumiko Tabuchi, Kenji Doi, Shouko Oota, Michiyoshi Sato, Toshiyuki Dohi, Hiroshi Ochi, Shinichiro Suyama, Yasuaki Mino; [Shikoku district] Masaaki Hattori, Masahiro Iwamoto, Kazumi Tsuzaki, Nobuo Matsuoka, Katsuyuki Fukuta, Toshihiro Goto, Sadanori Takeda, Shin Kimoto, Kiyonobu Tanaka, Motofumi Maguchi, Hiroshi Fukuda; [Kyushu district] Shoji Arihiro, Yuichirou Nakamura, Hideo Ikeda, Sohichi Uekihara, Yutaka Horio, Kazuki Takeshima, Koji Shiga, Shuji Inoue, Seiji Nishi, Yoshiaki Hayashi, Akito Sato, Nobuhisa Fukumoto, Yoshinao Uezu, Kensuke Matsushima.

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Review Article

Genetic polymorphisms of methylenetetrahydrofolate reductase and colorectal cancer and adenoma

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

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

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

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

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

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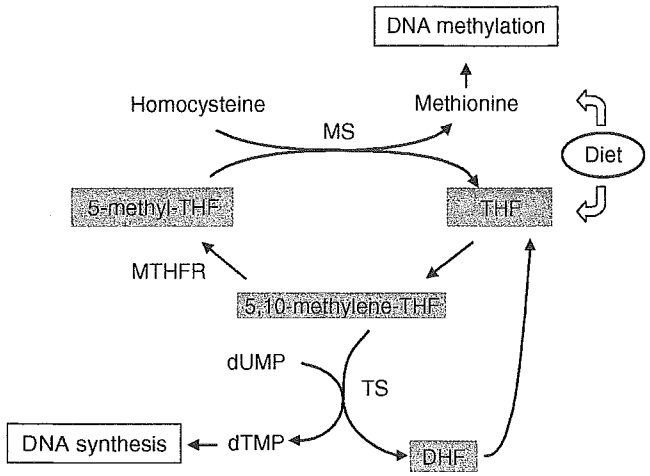

Fig. 1. Schematic presentation of folate metabolism in relation to DNA methylation and thymidylate synthesis. THF, tetrahydrofolate; MS, methionine synthase; TS, thymidylate synthase; dUMP, deoxyuridine monophosphate (deoxyuridylate); dTMP, deoxythymidine monophosphate (deoxythymidylate). The *MTHFR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

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

MTHFR polymorphisms and colorectal cancer risk

C677T polymorphism

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

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

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

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

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

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