

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 deoxyuridylylate to thymidylylate. Insufficient thymidylylate 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 μ L containing 0.5 U *Taq* and 1 μ L template DNA with a concentration of approximately 50–150 ng/ μ L. The *MTHFR C677T* genotype was determined, as described by Frosst *et al.*,⁽⁷⁾ using primers 5'-TGAAG GAGAA GGTGT CTGCC GGA-3' (sense) and 5'-AGGAC GGTGC GGTGA GAGTG-3' (antisense). The PCR product was digested with *Hinf*I, 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'-CAAAAT 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

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

Genotype	Cases		Controls		Crude OR	
	<i>n</i>	%	<i>n</i>	%	Adjusted OR	(95% CI) [†]
<i>MTHFR</i> 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)
<i>ALDH2</i>						
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.

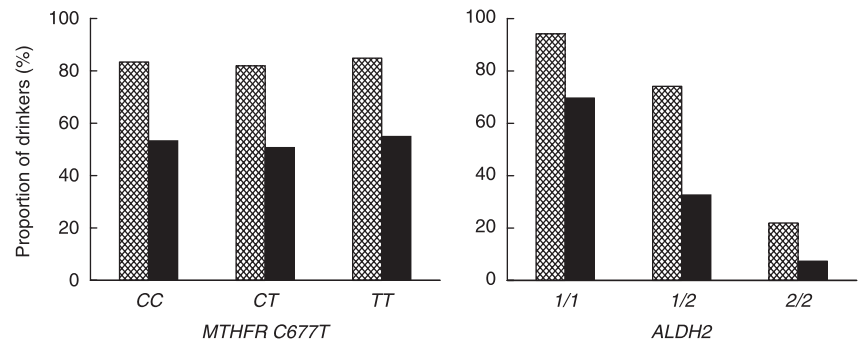


Fig. 1. Proportions (%) of current alcohol drinkers (hatched bar) and those consuming ≥ 30 mL alcohol per day (black bar) according to *MTHFR* C677T and *ALDH2* genotypes.

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) [‡]	
<i>MTHFR C677T</i>					
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)	
<i>ALDH2</i>					
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

<i>MTHFR C677T</i>	<i>ALDH2*1/1</i>		<i>ALDH2*1/2</i>		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

<i>MTHFR C677T</i>	<i>ALDH2*1/1</i>		<i>ALDH2*1/2</i>		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).

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* heterozygotes 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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