like that combined grilled/stir-fried meat and fish intake was a better surrogate for HCA intake.

HCA intake was not calculated from 28 days DR in this study because more than 130 kinds of fish are described in the Standard Tables of Food Composition in Japan [27], and it is difficult to develop the HCA database of fish. Although a database for HCA content has recently been developed for 297 food items [11], most food items have not been analyzed in Japan. This is because meat size or thickness and cooking conditions such as cooking temperature and time in Japan are different from those in the West. These also make it difficult to calculate HCA intake in meat.

On the one hand, the PhIP level in hair of the subjects in the present study (178–3674 pg/g hair) was lower than that of a report in the USA (500–50,000 pg/g hair) [16]. However, significant relationships between the PhIP level in hair and grilled/stir-fried meat and fish intake were observed. Only the PhIP level was compared with grilled/stir-fried meat and fish intake in the present study. However, because PhIP content was correlated with other HCA content in many foods [11], the PhIP level in hair could have been a biochemical indicator of not only PhIP intake but also other HCA intake.

Intake of cruciferous vegetables has been shown to reduce the excretion of PhIP in urine, and urinary mutagenicity was elevated [28,29]. However, the effect of cruciferous vegetable consumption on the PhIP level in human hair has not been reported. Since cruciferous vegetable intake has not been estimated, we could not examine the confounding effect of this factor in the present study. The relation between the PhIP level in hair and grilled/stir-fried meat and fish intake could be attenuated by the cruciferous vegetable intake.

Hardly any subject was a great eater of the burnt fish portion in the present study. Therefore, the PhIP level in hair and intake of the burnt portion may not have been correlated. In Japan, the pan-fried method of grilled/stir-fried meat frequently refers to short cooking time and not high meat temperature. Therefore, no difference in the present study was found between the PhIP level in the hair of a person who consumed well-done grilled/stir-fried meat out of preference and those who did not. Therefore, the PhIP level in hair and frequency of well-done grilled/stir-fried meat intake may also not have been correlated.

Melanin has a demonstrated affinity for HCAs in hair and is important for the uptake of HCAs in hair [16]. In our previous report, a positive correlation was observed between PhIP levels in hair (pg/g hair) and the melanin content in hair (μg/g hair) ($r = 0.45$, 95% CI = 0.04–0.86) [17]. However, trends of the mean PhIP levels in hair within tertile of corresponding grilled/stir-fried meat and fish intake estimated from DR were found to be no different between the crude levels (pg/g hair) and the levels per melanin content (ng/g melanin). There is a possibility that other polymers such as keratin may bind PhIP [30].

In conclusion, this study has shown that the PhIP level in hair could be used as a biochemical indicator of dietary intake of HCAs. These findings enable us to assess the relative validity of dietary HCA intake estimated from FFQ in comparison with the level in human hair.

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Folate, Vitamin B_6, Vitamin B_{12}, and Vitamin B_2 Intake, Genetic Polymorphisms of Related Enzymes, and Risk of Colorectal Cancer in a Hospital-Based Case-Control Study in Japan

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Abstract: *We conducted a case-control study to investigate the association of nutrient intake involved in the one-carbon pathway of folate for DNA methylation and DNA synthesis and the related enzyme genetic polymorphisms with colorectal cancer. Cases were 107 patients newly diagnosed with colorectal cancer. Controls were 224 subjects matched with cases by sex, age, and residential area. Nutrient intake was assessed by a self-administered, semiquantitative food-frequency questionnaire. Four genetic polymorphisms—*MTHFR *C677T and A1298C,* MTRR *A66G, and* ALDH2 *Glu487Lys—were determined using blood samples. Odds ratios were calculated using conditional logistic regression analysis adjusted for smoking, alcohol consumption, body mass index, and dietary fiber intake. Although folate intake was inversely associated with colorectal cancer, this association was attenuated after further controlling for dietary fiber intake. Neither vitamin B_6, vitamin B_{12}, nor vitamin B_2, nor any genetic polymorphism was significantly associated with colorectal cancer.* MTRR *polymorphism interacted with the association of folate (*P *for interaction = 0.04) or vitamin B_6 (*P *for interaction = 0.02) with colorectal cancer, although the other polymorphisms did not interact with any nutrient intake. In conclusion, the study did not support the existing hypothesis of gene–nutrient interaction in colorectal carcinogenesis.*

Introduction

Folate provides the one-carbon groups in the synthesis of thymidilates and the methylation of DNA and protein (1,2). Folate deficiency is assumed to cause uracil misincorporation, leading to DNA instability (3), and a retarded DNA repair for oxidative or alkylating damage, which has been implicated in the development of cancer (4). Such folate insufficiency can also lead to global (5) and proto-oncogenic DNA hypomethylation (6), resulting in human carcinogenesis including the large bowel.

Vitamins B_6 and B_{12} are involved in this folate metabolism pathway (2,7). Vitamin B_6 works as a cofactor for serine hydroxymethyltransferase, which catalyzes the formation of glycine and 5,10-methylenetetrahydrofolate from serine and tetrahydrofolate. This is an enzyme related to folate metabolism. Moreover, vitamin B_6 works as a cofactor for cystathionine-β-synthase, which catalyzes the conversion of homocysteine to cystathionine, a pathway that competes with the remethylation of homocysteine by methionine synthase to methionine. Vitamin B_{12} is a cofactor of methionine synthase and is important for maintaining adequate intracellular levels of methionine.

Vitamin B_2 (riboflavin) is the precursor for flavin adenine dinucleotide, the cofactor for methylenetetrahydrofolate reductase (*MTHFR*). The combination of vitamin B_2 intake and the genetic polymorphism C677T of *MTHFR* possibly affects folate metabolism, leading to colorectal carcinogenesis (8,9).

MTHFR metabolizes 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate and provides methyl groups to DNA methylation via the remethylation of homocysteine. This enzyme has a genetic polymorphism of C to T in the 677th base pair that causes a substitution of codon 222 alanine to valine and leads to lower activity of the enzyme than with no T variant (10). Previous studies revealed that the TT genotype (11,12) or the TT genotype with higher plasma folate (13,14) was inversely associated with colorectal cancer.

MTHFR has another common genetic polymorphism of A to C in the 1,298th base pair that causes a substitution of codon 429 glutamate to alanine. This polymorphism also

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leads to lower activity of the enzyme (15). Although this polymorphism was associated with a decreased risk of colorectal cancer (12), the evidence on the interaction with folate is sparse and inconsistent (16–19).

Methionine synthase reductase (*MTRR*) maintains methionine synthase in an active form. The methionine synthase is dependent on vitamin B_{12}. *MTRR* also has a common genetic polymorphism of A to G in the 66th base pair that causes a substitution of codon 22 isoleucine to methionine (20). This polymorphism may modify the effect of folate, vitamin B_6, or vitamin B_{12} to colorectal cancer (16), although the function of the polymorphism is still unknown.

Alcohol consumption was associated with folate malabsorption and folate deficiency (21,22). Folate deficiency was associated with a more-increased risk of colorectal cancer in drinkers (23–26), and drinkers were not associated with the decreased risk of colorectal cancer even if they had a high blood level of folate (14). Aldehyde dehydrogenase 2 (*ALDH2*) is one of the key enzymes of alcohol metabolism, and its genetic polymorphism of codon 487 glutamate to lysine broadly exists in Orientals including Japanese (27). Such a polymorphism exerts low activity of *ALDH2* (28) and causes high blood levels of acetaldehyde (29). The acetaldehyde possibly induces cleavage of folate (22,30). Therefore, the *ALDH2* genetic polymorphism may interact with folate in relation to colorectal cancer. However, to our knowledge, such interaction has never been investigated.

The folate and *MTHFR* C677T polymorphism interaction with colorectal cancer has been examined quite extensively, and evidence has been accumulated (12,31). In particular, evidence on the interaction between plasma folate and this polymorphism (13,14) has been accumulated. However, the interaction between other nutrients including vitamin B_6, vitamin B_{12}, and vitamin B_2 and other related enzyme genetic polymorphisms such as *MTHFR* A1298C (16–19), *MTRR* A66G (16), and *ALDH2* Glu487Lys has not yet been sufficiently investigated. These single nucleotide polymorphisms (SNPs) were selected for investigation because they related to the metabolism of folate and alcohol and were nonsynonymous SNPs in the coding region.

We investigated the association of folate, vitamin B_6, vitamin B_{12}, vitamin B_2, and genetic polymorphisms of related enzymes with colorectal cancer and also examined the interaction between these nutrients and genetic polymorphisms in relation to colorectal cancer risk in a multicenter, hospital-based case-control study.

Materials and Methods

Study Subjects

A hospital-based case-control study of gastrointestinal cancer was conducted between October 1998 and March 2002 at four hospitals in Nagano Prefecture, Japan (32,33). Eligible cases were colorectal cancer patients aged 20–74 yr who had been newly diagnosed during the survey at those hospitals. Consequently, we collected 121 colorectal cancer cases. No patient refused to participate in our study. We selected controls from medical checkup examinees in the four hospitals. Eligible healthy controls were those who were confirmed to have no cancer by the medical checkup, which included upper gastrointestinal endoscopy or X-ray, fecal occult blood test, or abdominal ultrasound, and, if screened in this medical checkup, confirmed by a subsequent detailed checkup. Two controls were matched for each case by sex, age (within 3 yr), and residential area during the study period in the same hospitals. Some cases had only one or more than two controls by way of exception. Although 249 potential controls were selected, 2 duplicate subjects enrolled were excluded. Of 247 controls, 2 individuals refused to participate in this study. Thus, 245 controls participated (participation rate = 99%) as matched controls. All cases were histopathologically confirmed according to the General Rules for Clinical and Pathological Studies on Cancer of the Colon, Rectum and Anus (34). We obtained written informed consent from all cases and controls. This study was approved by the Institutional Review Board of the National Cancer Center, Tokyo, Japan.

Exposure Assessment

We asked study subjects to answer a self-administered questionnaire that included general characteristics, such as age, sex, occupation, personal medical history, family history of disease, including colorectal adenoma and cancer, smoking and drinking habits, vitamin supplement use, and dietary habits. Their habitual consumption of foods and beverages was assessed with a 141-item, semiquantitative food-frequency questionnaire (FFQ). Subjects reported their average frequency of consumption and average portion size for those items during the past year. If subjects had any present symptoms, they provided their dietary habits for the year before the onset of symptoms. The mean daily consumption of energy and nutrients including folate, vitamin B_6, vitamin B_{12}, and vitamin B_2 was calculated using the food composition table developed for this FFQ based on the *Standard Tables of Food Composition in Japan*, 5th revised edition (35,36). The most important dietary sources were rice, spinach, and green tea for folate; rice, tuna, potatoes, bananas, and beer (only in men) for vitamin B_6; pacific saury, salted salmon roe, squid, mackerel, and clam for vitamin B_{12}; and egg, milk, rice, and green tea for vitamin B_2. Vitamin supplements were not included in the nutrient intake estimation because of no detailed information on B vitamins. Vitamin-enriched rice was included to calculate vitamin B_2 intake. The estimated consumption was validated with 14- or 28-day dietary records (DRs) and biomarkers in a prior study (37). The deattenuated rank correlation coefficients between FFQ and DR were as follows: 0.57 for folate, 0.59 for vitamin B_6, and 0.52 for vitamin B_{12} in men; 0.47 for folate, 0.63 for vitamin B_6, and 0.58 for vitamin B_{12} in women (unpublished data). The validity as rank correlation of vitamin B_2 intake was 0.34 in men and 0.45 in women (38).

Genotyping

Blood samples from subjects were collected at the same time as the questionnaire, and a buffy coat was preserved at –80°C until analysis. We determined four single-nucleotide polymorphisms (SNPs) in the following three genes: *MTHFR*, *MTRR*, and *ALDH2*. We used the MassARRAY (39,40) to measure SNPs in *MTHFR* and *ALDH2* and used the TaqMan® SNP Genotyping Assay of Applied Biosystems to measure an SNP in *MTRR* (Assay ID, C-3068176-10; dbSNP ID, rs1801394) (41). All four SNPs caused amino acid substitutions: codon 222 alanine to valine (677 C to T) and codon 429 glutamate to alanine (1298 A to C) in *MTHFR*; codon 22 isoleucine to methionine (66 A to G) in *MTRR*; and codon 487 glutamate (**1*) to lysine (**2*) in *ALDH2*.

Statistical Analysis

We excluded three non-adenocarcinoma cases (mucinous adenocarcinoma, squamous cell carcinoma, and final diagnosis as adenoma) and six matched controls. We then limited the study subjects to sufficient-blood sample donors (14 subjects were excluded) and those whose genotyping data were available. We failed to determine the genotypes of 12 samples because of an inadequate volume of abstracted DNA. This left 107 adenocarcinoma cases and 224 matched controls of analysis.

Characteristics of cases and controls were compared and tested by the Mantel-Haenszel test using matched-pair strata. We estimated odds ratios (ORs) and 95% confidence intervals (CIs) of colorectal cancer for folate, vitamin B_6, vitamin B_{12}, related-enzymatic SNPs, and the joint effect between nutrients and genotypes with the conditional logistic regression model on the matched case-control pairs. Nutrient intake was adjusted for total energy intake with the residual model (42) and divided into tertile categories based on the control subjects. In addition to controlling for sex, age, and hospitals in the statistical model, covariates for the adjustment of OR were smoking (never, 1–30 pack-years, 30 pack-years or more), alcohol consumption (never, past, current), body mass index (kg/m^2; tertiles based on controls), and total dietary fiber intake (tertiles based on controls, energy-adjusted using the residual method). We selected these confounding factors after checking whether they changed the ORs of the studied vitamins when entered in the statistical model (43). Other factors such as family history of colorectal cancer and red meat intake were not included in the final statistical model because these factors did not change the association of the studied vitamins with colorectal cancer when entered in the statistical model. To investigate whether related genotypes modified the effect of folate, vitamin B_6, vitamin B_{12}, or vitamin B_2, we mainly divided the subjects into individuals with variant (or mutant) alleles and those with two wild alleles and assessed the different effects of these nutrients by the genotype. *MTHFR* C677T genotypes were divided into homozygous mutant and others as same division as in most of the previous studies (13,14). Each different effect by the genotypes was tested with the log-likelihood ratio test using interaction terms between folate, vitamin B_6, vitamin B_{12}, or vitamin B_2 and genotypes (44). *P* values for all statistical tests were evaluated using the two-sided test with 0.05 as the significance level. All statistical analyses were conducted using the SAS program (SAS Institute, Cary, NC) (45).

Results

Folate and vitamin B_{12} intake were not statistically different between cases and controls (Table 1). Vitamin B_6 intake was higher in controls than in cases. Control subjects took in

Table 1. Characteristics of Cases and Controls[a]

	Unit	Case	Control	P^b
Number		107	224	
Sex (matching factor), men (%)		66 (62)	141 (63)	
Age (matching factor), mean (SD)	years	60 (9)	60 (9)	
Smoking, current (%)		25 (23)	43 (19)	0.41
Alcohol consumption, current (%)		58 (54)	151 (67)	0.013
Alcohol consumption, mean (SD)	g/day	19.8 (29.6)	17.7 (28.2)	0.57
BMI, mean (SD)	kg/m^2	22.8 (3.0)	23.7 (2.9)	0.032
Folate, mean (SD)	μg/day	417 (185)	439 (166)	0.14
Vitamin B_6, mean (SD)	mg/day	1.5 (0.3)	1.6 (0.3)	0.037
Vitamin B_{12}, mean (SD)	μg/day	9.3 (3.9)	9.9 (4.8)	0.20
Vitamin B_2, mean (SD)	mg/day	1.7 (0.5)	1.7 (0.5)	0.48
Total dietary fiber, mean (SD)	g/day	13.8 (6.5)	14.9 (6.3)	0.047
Red meat intake, mean (SD)	g/day	44.4 (32.4)	41.7 (23.5)	0.62
Total energy intake, mean (SD)	kcal/day	2,156 (735)	2,127 (755)	0.93
Family history of colorectal cancer (%)		15 (14)	20 (9)	0.22
Vitamin supplement use (%)		15 (14)	35 (16)	0.98

a: Abbreviations are as follows: SD, standard deviation; BMI, body mass index.
b: *P* for Mantel-Haenszel test with matched-pair strata.

more dietary fiber than case subjects. Body mass indices and percentage of current drinkers in cases were lower than in controls.

There were nonsignificantly inverse associations of *MTHFR* 677TT and 1298CC and *MTRR* 66GG with colorectal cancer (Table 2). *ALDH2* polymorphism showed a slight but not significantly increased risk of colorectal cancer.

Multivariate OR for folate, vitamin B_6, vitamin B_{12}, or vitamin B_2 was not statistically significant (Table 3). ORs for folate intake were 0.61 (95% CI = 0.32–1.1) for the second tertile and 0.55 (95% CI = 0.25–1.2) for the highest tertile (P for trend = 0.11) before being adjusted for total dietary fiber intake. After adjustment for dietary fiber intake, OR for the highest folate intake nonsignificantly increased (OR = 1.3; 95% CI = 0.49–3.4).

Statistical interaction (P for interaction = 0.04) was detected between folate intake and the *MTRR* polymorphism (Table 4). However, no linear trend was obtained in each polymorphism stratum. The association of folate with colorectal cancer did not differ by any genetic polymorphism of *MTHFR*.

Vitamin B_6 was associated with a decreased risk of colorectal cancer only in wild-type *MTRR* (Table 4). In heterozygous and homozygous mutant types of *MTRR*, a decreased risk was not observed for the higher intake of vitamin B_6 (P for interaction = 0.02). The highest tertile of vitamin B_6 intake was associated with a nonsignificant decreased risk only in the *MTHFR* 677TT genotype (OR = 0.39; 95% CI = 0.078–1.9), although the interaction was not statistically significant (P for interaction = 0.18). Two *MTHFR* polymorphisms did not statistically interact with vitamin B_6 intake in relation to colorectal cancer. Further, we tested the statistical interaction among folate intake, vitamin B_6 intake, and *MTRR* genotype. The interaction among these three variables was not significant (P for interaction = 0.48). The interaction between folate and the *MTRR* genotype was more important (P for interaction = 0.21) than that between vitamin B_6 and the *MTRR* genotype (P for interaction = 0.91) in this statistical model.

Table 2. Odds Ratios and 95% Confidence Intervals of Colorectal Cancer for Genotypes of Related Enzymes[a]

Gene	wt/wt	wt/mt	mt/mt	Trend P	mt[b]
MTHFR C677T	1.0 (reference)	0.75 (0.44–1.3)	0.79 (0.41–1.5)	0.44	0.95 (0.55–1.6)[c]
	32/51[d]	49/114	25/57		
MTHFR A1298C	1.0 (reference)	1.0 (0.58–1.7)	0.35 (0.040–3.0)	0.59	0.92 (0.54–1.6)
	73/156	32/63	1/5		
MTRR	1.0 (reference)	1.2 (0.72–1.9)	0.79 (0.27–2.3)	0.88	1.0 (0.70–1.5)
	58/128	44/82	5/14		
ALDH2	1.0 (reference)	1.1 (0.66–1.9)	1.2 (0.49–2.9)	0.60	1.1 (0.69–1.9)
	61/137	36/72	9/15		

a: Odds ratios on matched pair by matching factors. Abbreviations are as follows: wt, wild type; mt, mutant type.
b: wt/mt and mt/mt versus wt/wt.
c: mt/mt versus wt/wt and wt/mt.
d: Number of cases/number of controls.

Table 3. Odds Ratios[a] and 95% Confidence Intervals of Colorectal Cancer for Folate, Vitamin B_6, Vitamin B_{12}, and Vitamin B_2 Intake

Nutrient	Tertile 1	Tertile 2	Tertile 3	Trend P
Folate, mean (μg/day)	<343	343–484	485+	
	1.0 (reference)	1.0 (0.49–2.1)	1.3 (0.49–3.4)	0.62
	44/71[b]	32/78	31/75	
Vitamin B_6, mean (mg/day)	<1.46	1.46–1.74	1.75+	
	1.0 (reference)	1.1 (0.57–2.1)	0.88 (0.41–1.9)	0.77
	47/74	36/76	24/74	
Vitamin B_{12}, mean (μg/day)	<7.3	7.3–11.1	11.2+	
	1.0 (reference)	0.91 (0.48–1.7)	1.1 (0.55–2.2)	0.77
	36/72	37/77	34/75	
Vitamin B_2, mean (mg/day)	<1.49	1.49–1.84	1.85+	
	1.0 (reference)	0.87 (0.42–1.8)	1.1 (0.52–2.5)	0.64
	44/74	27/73	36/77	

a: Odds ratios on matched pairs by matching factors and adjusted for smoking (never, <30 pack-years, 30 pack-years or more), alcohol consumption (never, past, current), body mass index (tertiles based on controls), and total dietary fiber intake (tertiles based on controls).
b: Number of cases/number of controls.

Table 4. Odds Ratios[a] and 95% Confidence Intervals of Colorectal Cancer for the Combination Between Folate, Vitamin B_6, Vitamin B_{12}, or Vitamin B_2 Intake and Genotypes of Related Enzymes

Gene		Tertile 1	Tertile 2	Tertile 3	P^b
Folate					
MTHFR C677T	CC+CT	1.0 (reference)	1.1 (0.46–2.4)	1.4 (0.48–3.9)	
		33/53[c]	23/57	25/55	
	TT	1.2 (0.42–3.2)	1.2 (0.39–3.7)	1.2 (0.30–4.5)	0.91
		10/17	9/20	6/20	
MTHFR A1298C	AA	1.0 (reference)	0.82 (0.34–2.0)	1.1 (0.37–3.4)	
		30/48	21/54	22/54	
	AC+CC	0.83 (0.31–2.2)	1.3 (0.44–3.8)	0.93 (0.26–3.4)	0.63
		14/23	11/24	8/21	
MTRR A66G	AA	1.0 (reference)	1.8 (0.70–4.6)	1.1 (0.36–3.5)	
		25/46	20/38	13/44	
	AG+GG	1.3 (0.56–3.1)	0.71 (0.27–1.8)	2.1 (0.71–6.5)	0.043
		19/25	12/40	18/31	
Vitamin B_6					
MTHFR C677T	CC+CT	1.0 (reference)	1.1 (0.51–2.3)	1.2 (0.50–2.7)	
		32/57	27/56	22/52	
	TT	1.7 (0.62–4.6)	1.2 (0.45–3.5)	0.39 (0.078–1.9)	0.18
		14/16	9/20	2/21	
MTHFR A1298C	AA	1.0 (reference)	1.4 (0.64–3.2)	1.1 (0.43–2.8)	
		32/54	26/52	15/50	
	AC+CC	1.6 (0.61–4.1)	0.96 (0.35–2.7)	1.2 (0.39–3.6)	0.45
		15/20	9/24	9/24	
MTRR A66G	AA	1.0 (reference)	0.49 (0.20–1.2)	0.59 (0.23–1.5)	
		30/37	14/48	14/43	
	AG+GG	0.44 (0.19–1.02)	1.1 (0.51–2.6)	0.62 (0.22–1.7)	0.021
		17/37	22/28	10/31	
Vitamin B_{12}					
MTHFR C677T	CC+CT	1.0 (reference)	1.1 (0.51–2.3)	1.4 (0.62–3.2)	
		22/53	31/57	28/55	
	TT	1.7 (0.64–4.5)	0.81 (0.25–2.6)	1.0 (0.32–3.3)	0.43
		13/18	6/20	6/19	
MTHFR A1298C	AA	1.0 (reference)	0.81 (0.38–1.8)	0.99 (0.41–2.4)	
		27/48	26/60	20/48	
	AC+CC	0.66 (0.23–1.9)	1.1 (0.38–3.1)	1.1 (0.40–2.9)	0.60
		8/24	11/17	14/27	
MTRR A66G	AA	1.0 (reference)	1.4 (0.55–3.5)	1.3 (0.49–3.2)	
		16/42	23/42	19/44	
	AG+GG	1.5 (0.60–3.7)	0.88 (0.35–2.2)	1.6 (0.59–4.5)	0.40
		20/30	14/35	15/31	
Vitamin B_2					
MTHFR C677T	CC+CT	1.0 (reference)	1.0 (0.45–2.4)	1.3 (0.56–3.1)	
		31/54	22/51	28/60	
	TT	1.4 (0.51–3.8)	0.77 (0.23–2.5)	1.3 (0.39–4.3)	0.74
		12/19	5/22	8/16	
MTHFR A1298C	AA	1.0 (reference)	0.59 (0.24–1.4)	1.2 (0.49–3.0)	
		33/54	14/52	26/50	
	AC+CC	0.56 (0.19–1.6)	1.5 (0.55–3.8)	0.91 (0.32–2.6)	0.093
		10/20	13/21	10/27	
MTRR A66G	AA	1.0 (reference)	0.90 (0.36–2.2)	1.2 (0.48–2.9)	
		24/43	13/41	21/44	
	AG+GG	1.1 (0.49–2.5)	0.91 (0.35–2.3)	1.2 (0.45–3.3)	0.99
		20/31	14/32	15/33	

a: Odds ratios on matched pairs by matching factors and adjusted for smoking (never, <30 pack-years, 30 pack-years or more), alcohol consumption (never, past, current), body mass index (tertiles based on controls), and total dietary fiber intake (tertiles based on controls).

b: *P* for interaction.

c: Number of cases/number of controls.

Table 5. Odds Ratios[a] and 95% Confidence Intervals of Colorectal Cancer for the Combination Between Folate and Alcohol Consumption and Genetic Polymorphisms of Aldehyde Dehydrogenase

		Folate Intake			
		Tertile 1	Tertile 2	Tertile 3	P^b
Alcohol consumption	Never	1.0 (reference)	0.64 (0.13–3.2)	0.95 (0.21–4.4)	
		8/6[c]	10/22	21/36	
	Past + current	0.40 (0.12–1.4)	0.39 (0.11–1.4)	0.39 (0.084–1.8)	0.83
		26/65	22/56	10/39	
ALDH2	**1*1*[d]	1.0 (reference)	1.3 (0.54–3.1)	1.7 (0.53–5.2)	
		23/49	21/45	17/43	
	**1*2 +*2*2*[d]	1.4 (0.52–3.7)	0.93 (0.31–2.8)	1.2 (0.37–3.8)	0.50
		21/22	11/33	13/32	

a: Odds ratios on matched pairs by matching factors and adjusted for smoking (never, <30 pack-years, 30 pack-years or more), body mass index (tertiles based on controls), and total dietary fiber intake (tertiles based on controls).
b: *P* for interaction.
c: Number of cases/number of controls.
d: **1*, wild-type glutamate allele; **2*, mutant-type lysine allele.

No statistical interaction was observed in the combination between vitamin B_{12} or vitamin B_2 intake and any genotype of the related enzymes (Table 4).

There was no interaction between folate intake and alcohol consumption or the *ALDH2* genetic polymorphism (Table 5).

Discussion

We investigated the association of folate, vitamin B_6, vitamin B_{12}, and vitamin B_2 and genotypes of related enzymes with colorectal cancer. Our results suggested that the genetic polymorphism of *MTRR* may interact with folate and vitamin B_6 in relation to colorectal cancer.

Le Marchand et al. (16) reported that the *MTRR* A66G polymorphism was associated with colorectal cancer and did not interact with any nutrient, including folate, vitamin B_6, vitamin B_{12}, or vitamin B_2. The result of the present study suggested that higher vitamin B_6 intake may be useful only for wild-type *MTRR*. Although the folate and *MTRR* polymorphism interaction with colorectal cancer incidence was statistically significant, the effect of the interaction was difficult to interpret. The function of this polymorphism of *MTRR* is still unknown, and evidence on this polymorphism needs to be accumulated in colorectal cancer epidemiology as well as the experimental study area on carcinogenesis and prevention.

Our study did not suggest an inverse association of folate intake in the *MTHFR* variant genotype rather than the 677CC genotype. In fact, most previous studies failed to detect any statistical interaction between folate intake and the *MTHFR* C677T polymorphism (16–19,46–49). Previous studies revealed that the TT genotype with higher plasma folate was strongly inversely associated with risk (13,14). Both (13,14) were derived from the Physician's Health Study, which reported that age-adjusted OR for the folate rich and TT genotype was 0.32 (95% CI = 0.15–0.68) (13). In a different report, the age-adjusted OR for folate rich in TT genotype was 0.29 (95% CI = 0.12–0.73) compared with the CC/CT genotype (14). In addition, the inverse association of folate intake was not necessarily consistent among all studies (25,26, 50–53), possibly because other preventive factors may confound the association of folate intake with colorectal cancer. In fact, dietary fiber intake strongly confounded the association of folate intake with colorectal cancer in our study. Pearson's correlation coefficient was 0.83 between energy-adjusted folate and dietary fiber intake. This may be because the main food sources were the same between folate and dietary fiber, such as rice, cabbage, spinach, and Japanese white radish ("Daikon") (54). Folate appeared to be associated with colorectal cancer before being adjusted for dietary fiber intake. After the adjustment for fiber intake, however, the association of folate disappeared. That part of the evidence from some previous studies that showed the inverse association of folate intake may be due to a confounding with dietary fiber because not all studies considered fiber intake (25,52). Another possible reason is that folate intake may not reflect the absorbed and functional folate in the body. Folate absorption may be modified by alcohol consumption (21,22) and bowel microflora (30). Although folate intake calculated by the questionnaire correlated highly with folate intake estimated from DRs, plasma folate did not correlate highly with these records (rank correlation = 0.19). Therefore, the plasma folate concentration, which reflects the available folate, may more clearly demonstrate an interaction with a related genetic polymorphism rather than folate intake itself.

Many studies reported that folate deficiency was associated with the higher risk of colorectal cancer in drinkers than in nondrinkers (23–26). Our study did not confirm this evi-

dence. Moreover, our results showed that alcohol consumption decreased the risk of colorectal cancer, although not significantly. This was inconsistent with previous studies (13, 14,23,24). One possible reason was that some past drinkers were misclassified as never-drinkers. High-risk individuals may be included in the low-risk group, and the association appeared to be the converse direction of an alcohol-risk hypothesis. To our knowledge, our study is the first to evaluate the interaction between folate intake and the *ALDH2* polymorphism. The *ALDH2* genetic polymorphism exerts low enzyme activity (28) and causes high blood levels of acetaldehyde (29). Acetaldehyde possibly induces cleavage of folate (22,30) and inhibits its supply. Therefore, we hypothesized that subjects with the *ALDH2* mutant allele had a higher probability of folate deficiency than subjects without the mutant allele because acetaldehyde, which induces folate cleavage, may tend to stay in the blood circulation of those with the mutant allele after drinking. We expected that low folate with the *ALDH2* mutant allele would be associated with colorectal cancer more than that without the mutant allele. However, no significant interaction was obtained. Because the *ALDH2* genotype was associated with alcohol-drinking behavior (55), further larger studies should stratify study subjects by alcohol consumption to examine the interaction of the *ALDH2* genotype in the association between folate intake and colorectal cancer.

If study subjects had any symptoms, we asked both cases and controls to reply to us their existing dietary habits prior to their present symptoms. Because there were probably more study cases with present symptoms than controls, cases may have had to recall a more distant past than controls. Disease experience may also affect recall, and cases may more earnestly seek to recall dietary habits than controls. Recalling the more distant past or recalling it more earnestly in cases may lead to differential misclassification. Moreover, information on the distant past in cases may be more vague than in controls, leading to underestimation of the effects of nutrients. On the other hand, the more accurate information on dietary habits by the more earnest recall of cases than controls may result in overestimation of the effects of nutrients. We did not assess symptom-related changes in dietary habit. Our small sample size may limit the interpretation of our results. We may have failed to demonstrate the association and the evaluation of gene–nutrient interactions. Because almost all subjects participated in this study, nonresponse bias may be small. However, controls were selected from medical checkup examinees who were interested in their health and screening. Therefore, there may be a self-selection bias by the healthy volunteer effect. We intended to investigate another genetic polymorphism, methionine synthase A2756G. This enzyme is a member of the folate metabolic pathway, and this genetic polymorphism may modify the effect of folate or related nutrients on colorectal cancer (13,16). Although we performed *MTR* genotyping, we failed to obtain reliable data on this polymorphism.

In conclusion, the present study did not support the existing hypothesis of gene–nutrient interaction and inverse association of folate intake with colorectal cancer nor did it show any significant association of vitamin B_6, vitamin B_{12}, vitamin B_2, or related-enzyme genetic polymorphisms.

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Alcohol consumption and the risk of cancer in Japanese men: the Miyagi cohort study

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The objective of this study was to investigate the association between alcohol consumption and the risk of total cancer, and to estimate the proportion of total cancer attributable to drinking habit in Japanese men. From June through August 1990, a total of 21 201 Japanese men completed a self-administered questionnaire on various health habits, including alcohol consumption. During 153 389 person-years of follow-up through December 1997, we identified a total of 882 cases of cancer. We used Cox proportional hazards regression to estimate the relative risk of total cancer according to categories of alcohol consumption. The risk for total cancer was significantly higher in ex-drinkers than never-drinkers. There was a dose–response relationship between the amount of alcohol consumed and the risk of total cancer among current drinkers: multivariate RRs in reference to never-drinkers (95% confidence intervals (CI)) were 1.1 (0.8–1.3), 1.3 (1.0–1.7), and 1.3 (1.1–1.7) in current drinkers who consumed less than 22.8 g, 22.8–45.5 g, 45.6 g or more alcohol per day, respectively (*P* for trend <0.001). Estimated 17.9% (95% CI 3.1–30.5) of total cancer risk was attributable to drinking habit. In our findings, approximately 20% of the total cancer cases in Japanese men may be prevented by alcohol control. *European Journal of Cancer Prevention* 14:169–174 © 2005 Lippincott Williams & Wilkins.

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Keywords: Alcohol consumption, cancer, Japanese, population attributable fraction, prospective cohort study

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Introduction

Cancer is the leading cause of death among Japanese men, and it accounted for 34.3% of mortality (181 393 deaths) from all causes in 2001. Various lifestyle factors, such as diet, smoking and drinking, have been shown to play a role in the causation of cancer (Williams *et al.*, 1999).

Alcohol consumption has been associated with increased risk of cancer in several organs, and a panel of experts commissioned by the World Cancer Research Fund and the American Institute for Cancer Research in 1997 concluded that there is 'convincing' evidence that drinking increases the risk of cancer of the oral cavity, pharynx, oesophagus and liver, and 'probable' evidence that drinking increases the risk of cancer of the colon, rectum and breast (World Cancer Research Fund/American Institute for Cancer Research, 1997).

Several studies have shown a statistically significant association between excessive drinking and increased risk of cancer of the stomach (Hirayama *et al.*, 1989; Kato *et al.*, 1992), colon (Hirayama *et al.*, 1989; Otani *et al.*, 2003; Shimizu *et al.*, 2003), rectum (Hirayama *et al.*, 1989; Otani *et al.*, 2003; Shimizu *et al.*, 2003), liver (Kono *et al.*, 1986), oesophagus (Hirayama *et al.*, 1989; Kinjyo *et al.*, 1998), oral cavity (Hirayama *et al.*, 1989), and pharynx (Hirayama *et al.*, 1989) in the Japanese population. Three prospective studies have examined the association between alcohol drinking and the risk of total cancer, and found that excessive drinking consistently increased the risk of total cancer in the Japanese population (Kono *et al.*, 1986; Takezaki *et al.*, 1999; Tsugane *et al.*, 1999). However, these studies had several limitations: all of them used cancer mortality as endpoint (Kono *et al.*, 1986; Takezaki *et al.*, 1999; Tsugane *et al.*, 1999), two studies only controlled for age (Kono *et al.*, 1986; Takezaki *et al.*, 1999) and smoking habits (Kono *et al.*, 1986), and two studies have not been confirmed the validity and reliability of questionnaire assessment of alcohol consumption (Kono *et al.*, 1986; Takezaki *et al.*, 1999).

The objective of this study was to investigate the association between alcohol consumption and the risk of total cancer in Japanese men, and to estimate the proportion of total cancer attributable to drinking habit.

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Methods

Study cohort

We have reported the design of this prospective cohort study in detail elsewhere (Fukao *et al.*, 1995; Tsubono *et al.*, 2001; Nakaya *et al.*, 2003). Briefly, from June through August 1990, we delivered a self-administered questionnaire on various health habits to 25 279 men who were 40–64 years of age and lived in 14 municipalities of Miyagi Prefecture in Northern Japan. The questionnaires were delivered and collected at the subjects' residences by members of health promotion committees appointed by the municipal governments. Usable questionnaires were returned from 22 836 men, yielding a 91.9% response rate. The study protocol was approved by the institutional review board of the Tohoku University Graduate School of Medicine. We considered the return of a self-administered questionnaire signed by a subject to imply his consent to participate in the study.

Exposure data

The questionnaire assessed alcohol consumption by first asking if the subject was a never-, ex- or current drinker. Current drinkers were then asked about frequency of drinking (less than once per week, once or twice per week, 3 or 4 times per week, or 5 times or more per week), beverage type usually consumed (sake, spirits, beer, whisky, wine or other), and the amount at one occasion. We calculated from these data the amount of alcohol consumed per day in grams. The subjects were classified into five categories; never-drinkers, ex-drinkers, current drinkers who consumed less than 22.8 g alcohol per day, 22.8–45.5 g alcohol per day and 45.6 g or more alcohol per day. The traditional unit of sake, 1 *go* (180 ml) is the same as 22.8 g of alcohol, which also approximates two glasses of wine (200 ml) or two measures of spirits (50 ml) in terms of alcohol contents. We conducted a validation study for the questionnaire assessment of alcohol consumption in which 113 subjects in the study district provided four 3-day diet records in one year and then responded to the questionnaire (Ogawa *et al.*, 2003). Spearman's coefficient of correlation between the amounts of alcohol consumption consumed according to the questionnaire and the amounts consumed according to the diet records was 0.61, and the correlation between consumption measured by the two questionnaires administrated 12 months apart was 0.81.

Follow-up

We used population registries in the 14 municipalities to ascertain vital and residential status of the subjects from 1 June 1990 to 31 December 1997. We identified incident cases of cancer by means of computerized record linkage with the Miyagi Prefectural Cancer Registry covering the study area (Takano and Okuno, 1997).

Out of the 22 836 subjects who responded to the questionnaire, we excluded subjects who had incomplete responses in alcohol information ($n = 1243$). We also excluded 392 subjects who had had prevalent cancer according to self-reports on the questionnaire or records of the cancer registry. Consequently, 21 201 men with 882 incident cases of cancer remained for this analysis.

We counted person-years of follow-up for each subject from 1 June 1990, until the date of diagnosis of cancer, date of moving to outside the study municipalities, date of death, or the end of the study period (31 December 1997), whichever occurred first. A total of 153 389 person-years accrued. Follow-up of subjects who moved from the study municipalities was discontinued because of logistical limitations, and 737 subjects (3.5% of the analytic cohort) were lost to follow-up during the study period.

Statistical analysis

The Cox proportional-hazards regression was used to estimate relative risk of cancer and to adjust for potentially confounding variables, using the SAS PHREG procedure on the SAS version 8.2 statistical software package (SAS, Cary, NC, USA). Several cancer endpoints were used, including total cancer (882 cases), alcohol-associated cancers (308), and cancer at sites unassociated with drinking (567). Cancers of the colon (106), rectum (67), oesophagus (52), liver (48), oral cavity (19), and larynx (16) were regarded as being associated with alcohol consumption (World Cancer Research Fund/American Institute for Cancer Research, 1997). We also used as the endpoints the six individual cancer sites in which more than 40 incident cases were identified among the analytic cohort during the follow-up period, namely, the stomach (247 cases), lung (119), colon (106), rectum (67), oesophagus (52) and liver (48).

We considered the following variables as potential confounders: age in years, cigarette smoking (never smoked, smoked in the past, currently smoking 1–19 cigarettes per day, currently smoking 20–29 cigarettes per day, or currently smoking at least 30 cigarettes per day), education (in school until 15 years of age, 16–18, or 19 years or older); and consumption frequencies of spinach, carrot or pumpkin, tomato, orange, other fruits, and juice (less than 1 day per week, 1 or 2 days per week, 3 or 4 days per week, or daily). Because we observed similar results whether we used the categorical variables of smoking, the number of pack-years of smoking, or the number of the cigarettes currently smoked per day, we present the results with the categorical variables of smoking. We repeated all analyses after excluding each cancer cases diagnosed in the first 3 years of follow-up. *P*-values to test for linear trends were estimated using grams of alcohol consumed per day as a continuous variable with ex-drinkers excluded. All *P*-values were two-tailed.

The population attributable fraction (PAF) was calculated as pd × {RR − 1}/RR, where pd is the proportion of cases exposed to the risk factor, i.e. the proportion of ex- and current drinkers combined (Rockhill *et al.*, 1998). The 95% confidence intervals (CIs) of the PAFs were calculated by the formula of Greenland (Greenland, 1999). This formula is known to be more valid than the popular formula (RR − 1) × Pe/{1 + (RR − 1) × Pe}, where Pe is the proportion of source population exposed to the risk factor, when confounding variables exist (Rockhill *et al.*, 1998).

Results

The proportions of never-drinkers, ex-drinkers, current drinkers who consumed less than 22. 8 g, 22.8–45.5 g and 45.6 g or more alcohol per day were 16%, 8%, 23%, 18%, 35%, respectively. Table 1 compares the characteristics of the subjects according to drinking categories. Compared with never-drinkers, current drinkers were younger, more likely to be current smokers, less likely to consume daily oranges, other fruits, juice, carrot or pumpkin or tomato. Compared with never-drinkers, ex-drinkers were also older, more likely to be ex-smokers and less likely to consume oranges daily.

Table 2 shows the association between alcohol drinking and the risk of total cancer, alcohol-associated cancer and cancer at sites unassociated with drinking. The age-adjusted relative analysis showed that the risk of total cancer was significantly higher in current drinkers than in never-drinkers, and that the risk increased linearly with the amount of alcohol consumed. This finding remained basically unchanged after multivariate adjustment or exclusion of the subjects diagnosed with cancer during the first three years of follow-up. The multivariate-adjusted relative analysis showed significantly higher risk of alcohol-associated cancers in current drinkers than in never-drinkers, and that the risk increased linearly with the amount of alcohol consumed. The risk was 1.9-fold higher in current drinkers who consumed 45.6 g or more alcohol per day than in never-drinkers. Consumption of a moderate amount of alcohol (< 22.8 g/day) was not associated with a lower risk of alcohol-associated cancers. The linear increase in risk was more evident after exclusion of cancer cases diagnosed within the first 3 years of follow-up, when the risk for current drinkers who consumed less than 22.8 g alcohol per day was 1.7-fold higher than for never-drinkers. Alcohol consumption showed no significant association with the risk of cancer at sites unassociated with drinking.

Table 3 shows multivariate relative risks for the six major individual cancer sites according to drinking categories. Higher, but not significantly higher risk of alcohol-associated cancers (rectum, colon, oesophagus and liver) in current drinkers than never-drinkers was found (1.4–2.7). Remarkably, ex-drinkers had a higher risk of liver cancer and lung cancer than never-drinkers.

An estimated 17.9% (95% CI 3.1–30.5) of total cancer risk was attributable to drinking habit. Furthermore, an estimated 35.6% (95% CI 10.9–53.4) of alcohol-associated cancers risk was attributable to drinking habit.

We conducted stratified analyses according to 5-year age class, cigarette smoking, education and frequency of consumption of food items. However, the associations between alcohol consumption and the risk of total cancer were not remarkably modified by these variables (data not shown).

Discussion

This prospective cohort study investigated the association between alcohol consumption and the risk of cancer in Japanese men. The results showed that (1) the risk of total cancer in current drinkers was higher than in never-drinkers; (2) the risk of total cancer in ex-drinkers was higher than in never-drinkers; (3) total cancer risk

Table 1 Characteristics of the subjects according to alcohol consumption

Characteristics	Ex-drinkers	Never-drinkers	Current drinkers (g alcohol/day)			
			All	<22.8	22.8–45.5	≥ 45.6
No. of subjects	1594	3349	16258	4915	3907	7436
Age, years, mean ± SD	54.9 ± 7.2	52.5 ± 7.7	51.1 ± 7.5	50.7 ± 7.6	51.6 ± 7.7	51.2 ± 7.4
Cigarette smoking[a] (%)						
Never	13.2	27.4	17.4	25.7	18.0	11.6
Past	31.6	15.3	19.6	20.9	21.4	17.8
Current	55.2	57.4	63.0	53.3	60.6	70.6
Education, in school 19 years of age or older (%)	11.5	13.1	14.8	15.7	14.6	13.6
Daily dietary consumption (%)						
Orange	18.6	23.2	16.5	20.7	17.3	13.4
Other fruits	26.9	26.9	19.9	24.4	20.5	16.4
Juice	7.4	8.6	5.9	6.5	6.2	5.4
Spinach	21.7	19.8	20.2	20.7	21.0	19.4
Carrot or pumpkin	12.8	10.2	8.9	9.8	8.8	8.4
Tomato	16.5	14.0	12.7	14.1	13.1	11.6

[a]Because of rounding, not all percentages add to 100.

Table 2 Relative risk of total cancer, cancer sites associated with drinking, and cancer at sites unassociated with drinking by alcohol consumption[a]

	Ex-drinkers	Never-drinkers	Current drinkers (g alcohol/day)				*P* for trend
			All	<22.8	22.8–45.5	≥ 45.6	
Person-years	11 234	24 370	117 785	35 712	28 193	53 880	
Total cancer (882 cases)							
No. of cases	92	122	668	158	175	335	
Age-adjusted RR1	1.4 (1.0–1.8)	1.0	1.3 (1.1–1.6)	1.0 (0.8–1.3)	1.4 (1.1–1.7)	1.4 (1.2–1.8)	<0.001
Multivariate RR1	1.3 (1.0–1.8)	1.0	1.3 (1.0–1.5)	1.1 (0.8–1.3)	1.3 (1.0–1.7)	1.3 (1.1–1.7)	0.001
Multivariate RR2	1.5 (1.0–2.1)	1.0	1.3 (1.0–1.7)	1.1 (0.8–1.5)	1.3 (1.0–1.8)	1.4 (1.1–1.9)	0.003
Alcohol-associated cancers (308 cases)							
No. of cases	32	33	243	53	62	128	
Age-adjusted RR1	1.8 (1.1–2.9)	1.0	1.7 (1.2–2.5)	1.3 (0.8–2.0)	1.8 (1.2–2.7)	2.0 (1.4–3.0)	<0.001
Multivariate RR1	1.8 (1.1–2.9)	1.0	1.7 (1.1–2.4)	1.3 (0.8–2.0)	1.7 (1.1–2.6)	1.9 (1.3–2.8)	<0.001
Multivariate RR2	1.9 (1.0–3.6)	1.0	2.1 (1.3–3.3)	1.7 (1.0–2.9)	1.9 (1.1–3.3)	2.4 (1.5–4.0)	<0.001
Cancer at sites unassociated with drinking (563 cases)							
No. of cases	58	88	417	105	109	203	
Age-adjusted RR1	1.2 (0.9–1.7)	1.0	1.1 (0.9–1.4)	1.0 (0.7–1.3)	1.2 (0.9–1.6)	1.2 (0.9–1.6)	0.045
Multivariate RR1	1.2 (0.8–1.6)	1.0	1.1 (0.9–1.4)	1.0 (0.7–1.3)	1.2 (0.9–1.5)	1.1 (0.9–1.5)	0.23
Multivariate RR2	1.3 (0.9–1.9)	1.0	1.0 (0.8–1.4)	0.9 (0.7–1.3)	1.1 (0.8–1.5)	1.1 (0.8–1.5)	0.43

[a]The multivariate relative risk (RR) has been adjusted for age (in years); cigarettes smoking (never smoked, smoked in the past, currently smoking 1–19 cigarettes per day, or currently smoking 20–29, or 30 or more cigarettes per day); education (in school until age 15 years or younger, 16–18, or 19 years or older); daily consumption of orange, other fruits, juice, spinach, carrot or pumpkin, and tomato (less than 1 day per week, 1 or 2 days per week, 3 or 4 days per week, or daily). RR1 denoted the relative risk with all cases of cancer induced in the multivariate analysis, RR2 the relative risk with cases diagnosed in the first three years of follow-up excluded from the analysis. Alcohol-associated cancers include colon (106 cases), rectum (67), oesophagus (52), liver (48), oral cavity (19), and larynx (16). Values in parentheses are 95% confidence intervals.

Table 3 Multivariate relative risk of individual cancer sites according to alcohol consumption[a]

	Ex-drinkers	Never-drinkers	Current drinkers (g alcohol/day)			*P* for trend
			All	<22.8	≥ 22.8	
Stomach (247 cases)						
No. of cases	21	42	184	49	135	
Multivariate RR	0.9 (0.5–1.5)	1.0	1.0 (0.7–1.4)	1.0 (0.6–1.5)	1.0 (0.7–1.5)	0.83
Lung (119 cases)						
No. of cases	21	16	82	17	65	
Multivariate RR	2.3 (1.2–4.4)	1.0	1.2 (0.7–2.1)	1.0 (0.5–2.0)	1.3 (0.8–2.3)	0.30
Colon (106 cases)						
No. of cases	10	11	85	19	66	
RR	1.6 (0.7–3.8)	1.0	1.7 (0.9–3.3)	1.3 (0.6–2.8)	1.9 (1.2–3.7)	0.03
Rectum (67 cases)						
No. of cases	3	9	55	13	42	
Multivariate RR	0.6 (0.2–2.3)	1.0	1.4 (0.7–2.9)	1.2 (0.5–2.8)	1.5 (0.7–3.1)	0.23
Oesophagus (52 cases)						
No. of cases	4	4	44	4	40	
Multivariate RR	1.8 (0.4–7.1)	1.0	2.5 (0.9–7.1)	0.9 (0.2–3.5)	3.2 (1.1–8.9)	0.004
Liver (48 cases)						
No. of cases	10	3	35	11	24	
Multivariate RR	6.6 (1.8–24.2)	1.0	2.7 (0.8–8.9)	2.8 (0.8–10.1)	2.7 (0.8–8.9)	0.21

[a]The multivariate relative risk (RR) has been adjusted for age (in years); cigarette smoking (never smoked, smoked in the past, currently smoking 1–19 cigarettes per day, or currently smoking 20–29, or 30 or more cigarettes per day); education (in school until age 15 years or younger, 16–18, or 19 years or older); daily consumption of orange and other fruit juice, spinach, carrot or pumpkin, and tomato (less than 1 day per week, 1 or 2 days per week, 3 or 4 days per week, or daily). Values in parentheses are 95% confidence intervals.

increased linearly as the amount of alcohol consumption increased; (4) 17.9% of total cancer risk was attributable to drinking habit.

The results of this study showed that total cancer risk increased linearly with the amount of alcohol consumed, and that the risk of total cancer was significantly higher in excessive drinkers (45.6 g or more alcohol per day) than in never-drinkers. Three earlier studies examined the association between alcohol consumption and the risk of total cancer in Japanese men and consistently found that excessive drinking increased risk of total cancer (Kono *et al.*, 1986; Takezaki *et al.*, 1999; Tsugane *et al.*, 1999). A dose–response relationship between alcohol consumption and the risk of total cancer were inconsistent findings. More specifically, Kono *et al.* (1986) showed a linear association between alcohol consumption and the risk of total cancer. Tsugane *et al.* (1999) reported a J-shaped association between alcohol consumption and the risk of total cancer. Takezaki *et al.* (1999) showed linear association between alcohol consumption and the risk of total cancer.

Seven prospective cohort studies of the association between alcohol consumption and the risk of total cancer or alcohol-associated cancers have been conducted in the world excluding Japanese populations. Of these two studies found a linear association between alcohol consumption and the risk, and indicated that besides, moderate current drinkers began to increase in risk compared with the non drinkers (Thun *et al.*, 1997; Grønbæk *et al.*, 2000). Five studies found J-shaped or U-shaped associations, because of moderate current drinkers found decrease in risk compared with the non-drinkers (Farchi *et al.*, 1992; Goldberg *et al.*, 1994; Fuchs *et al.*, 1995; Maskarinec *et al.*, 1998; Renaud *et al.*, 1998).

The discrepancy between the present results and those of most previous studies showing a J-shaped or U-shaped association may be partly explained by the exclusion in this study of ex-drinkers from the reference category. In most previous studies, 'non-drinkers' comprised both never drinkers and ex-drinkers. In the present analysis, we considered ex-drinkers and never drinkers separately, and found that ex-drinkers had markedly higher risk of total cancer or alcohol-related cancer risk compared with never drinkers. Higher cancer incidence among ex-drinkers may be due to ill-health that had led them to quit drinking (Goodman *et al.*, 1995; Tsubono *et al.*, 2001). Studies of alcohol consumption and the risk of cancer may overestimate the lower risk in moderate drinkers if they did not separate never drinkers and ex-drinkers in the referent group.

Our study had several methodological advantages over previous studies examining the association between alcohol consumption and the risk of total cancer in Japan. First, our study used cancer incidence, rather than mortality, as an endpoint, which made it possible to distinguish whether alcohol consumption was related to cancer incidence, cancer survival, or both. Second, we controlled extensively for potentially confounding variables, such as smoking, education (a measure of socio-economic status), and diet (consumption of vegetables and fruits). Third, we established the validity and reliability for questionnaire assessment of alcohol consumption (Ogawa *et al.*, 2003).

Several studies have shown a statistically significant association between excessive drinking and increased risk of cancer of the stomach (Hirayama *et al.*, 1989; Kato *et al.*, 1992), colon (Hirayama *et al.*, 1989; Otani *et al.*, 2003; Shimizu *et al.*, 2003), rectum (Hirayama *et al.*, 1989; Otani *et al.*, 2003; Shimizu *et al.*, 2003), liver (Kono *et al.*, 1986), oesophagus (Hirayama *et al.*, 1989; Kinjyo *et al.*, 1998), oral cavity (Hirayama *et al.*, 1989) and pharynx (Hirayama *et al.*, 1989) in the Japanese population. No significantly higher risk of alcohol-associated cancers (rectum, colon, oesophagus and liver) in current drinkers than never-drinkers was found in our study (1.4–2.7), but our study may not have sufficient statistical power to detect small increases or decreases in risk of cancer at individual sites associated with alcohol consumption, because the number of cases of cancer sites was only modest to small (48–247 cases). Thus, our follow-up period and the number of cases of cancer were probably insufficient to evaluate the association between alcohol consumption and the risk of total cancer, alcohol-associated cancers, or major cancer sites, and we need further to estimate the associations in a future.

We concluded that the risk of total cancer was higher in ex- and current drinkers than in never-drinkers and that the risk increased linearly with the amount of alcohol consumed. Approximately 20% of the total cancer cases in Japanese men could be prevented by alcohol control.

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Fruit and vegetable consumption and risk of colorectal cancer in Japan: The Miyagi Cohort Study

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Abstract

Objective: Adequate fruit and vegetable intake has been suggested to protect against colorectal cancer. However, several recent prospective studies have reported no association. We therefore examined the association between fruit and vegetable intakes and the risk of colorectal cancer in a prospective cohort study in Japan.

Design: Between June and August 1990, 47 605 Japanese men and women completed a self-administered questionnaire, including a food-frequency questionnaire. We divided the subjects into quartiles based on their self-reported fruit and vegetable consumption. There were 165 colon cancer and 110 rectal cancer incidences identified during 7 years of follow-up, to the end of December 1997. We used Cox proportional hazards models to estimate the relative risk (RR) of developing colorectal cancer according to the level of fruit and vegetable consumption, applying adjustments for potential confounders.

Results: No statistically significant association was observed between fruit and vegetable consumption and the risk of colorectal cancer. The multivariate RR of colon cancer in the highest quartile of fruit and vegetable intake compared with the lowest was 1.13 (95% confidence interval (CI) 0.73–1.75), the RR for vegetables alone was 1.24 (95% CI 0.79–1.95) and that for fruit alone was 1.45 (95% CI 0.85–2.47). The corresponding multivariate RRs for rectal cancer were 1.12 (95% CI 0.67–1.89), 1.14 (95% CI 0.67–1.93) and 1.41 (95% CI 0.73–2.73).

Conclusions: We found no association between the consumption of fruit and vegetables and the risk of colorectal cancer among the Japanese population.

Keywords
Colorectal cancer
Food-frequency questionnaire
Prospective cohort study
Vegetables
Fruit

Colorectal cancer is a major cause of death in Western countries[1] and is also associated with high levels of mortality in Japan. In Japan, mortality from colorectal cancer increased rapidly between 1950 and 2001[2]. During this period, the age-adjusted mortality rate per 100 000 for colon cancer increased 4.9-fold in men (2.9 in 1950 vs. 14.2 in 2001) and 2.9-fold in women (3.3 in 1950 vs. 9.5 in 2001). The rate for rectal cancer increased 1.6-fold in men (5.6 in 1950 vs. 9.2 in 2001), but remained stable in women (4.2 in 1950, compared with 4.1 in 2001).

A comprehensive review by the World Cancer Research Fund in 1997 examined the association between diet and colorectal cancer[3]. Based on 22 case–control[4–25] and four prospective cohort[26–29] studies, this organisation concluded that there was 'convincing' evidence to indicate that diets rich in vegetables had a protective effect against colon and rectal cancers. The data on fruit were limited and inconsistent, and no judgement was possible. However, all recent prospective cohort studies[30–34] except one[35] have found no inverse association between fruit and vegetable intakes and the risk of colorectal cancer. Thus, evidence that vegetables and fruits may reduce the risk of colorectal cancer has not been established[36].

The objective of our study was to prospectively examine the association between fruit and vegetable intake and the risk of colorectal cancer in Japan, where dietary habits and genetic backgrounds differ considerably from those in previous cohort studies in Western countries. To our knowledge, no previous prospective cohort study has examined this association in any Asian country, including Japan.

Subjects and methods

Study cohort

The design of the present cohort study has been described in detail elsewhere[37]. Briefly, between June and August 1990, we delivered a self-administered questionnaire

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on various health habits to 51 921 subjects (25 279 men and 26 642 women) aged 40–64 years living in 14 municipalities of Miyagi Prefecture in rural northern Japan. Usable questionnaires were returned by 47 605 subjects (22 836 men and 24 769 women), yielding 91.7% response rate.

The study protocol was approved by the institutional review board of Tohoku University Graduate School of Medicine, and we considered return of a signed self-administered questionnaire to imply each subject's consent to participate in the study.

Dietary assessment

Dietary intake was assessed using a self-administered 40-item food-frequency questionnaire (FFQ) asking about the average frequency of consumption of several foods. For seasonal foods, the subjects were asked to report the intake frequency in high season. Five frequency categories were used for the majority of food items (almost never, 1–2 days per month, 1–2 days per week, 3–4 days per week, almost every day). We determined a portion size for each food item based on the median values observed in 12-day dietary records (DRs) collected from separate male and female sub-samples of the subjects. We calculated the weight of total vegetables consumed each day based on six items (green leafy vegetables, carrots and pumpkins, tomatoes, cabbage and lettuce, Chinese cabbage and *tsukemono* (pickles)). Similarly, total fruit intake was based on three items (oranges, other fruits and fresh fruit juices). The validity of fruit and vegetable intake was assessed by calculating Spearman correlation coefficients between the DRs and the FFQ for the relevant factors. Adjusted and deattenuated Spearman correlation coefficients were 0.59 for total fruit and vegetable intake, 0.50 for total vegetable intake and 0.59 for total fruit intake. In addition, adjusted Spearman correlation coefficients between two FFQs administered 1 year apart (which acted as a measure of reproducibility) were 0.67 for total fruit and vegetables, 0.55 for total vegetables and 0.72 for total fruit intake[38].

Follow-up

We identified incident cases of colon and rectal cancers that occurred in our study cohort from 1 June 1990 to 31 December 1997 by linkage of computerised records with the Miyagi Prefectural Cancer Registry, which covers the study area. We counted person-years of follow-up for each subject from 1 June 1990 until the date of diagnosis of colon or rectal cancer, the date of death, the date of moving out of the study area, or the end of the follow-up period (31 December 1997), whichever occurred first. The total numbers of person-years of follow-up accrued for the colon and rectal cancer analyses were 307 779 and 307 675, respectively.

Of the 47 605 subjects who responded to the questionnaire, we excluded subjects with a previous diagnosis of cancer ($n = 1110$) and those who had extreme levels of energy intake (below or above 5% of the range for all subjects; $n = 4660$). Consequently, 41 835 subjects (20 174 men and 21 661 women) with 165 colon cancers and 110 rectal cancers were included in the present analysis.

Statistical analysis

We carried out separate analyses for colon and rectal cancers. Consumption of total vegetables and of total fruit was grouped into quartiles. Energy adjustment of food intakes was performed using the residual method[39]. We used Cox proportional hazards regression to calculate the relative risk (RR) and 95% confidence interval (CI) for developing cancer, and to adjust for potentially confounding variables[40]. These analyses were performed using the SAS PHREG procedure on SAS version 8.2 statistical software (SAS Institute, Cary, NC, USA). The multivariate analyses were adjusted for sex, age (in years), smoking status (never, past, currently smoking 1–19 cigarettes daily, currently smoking 20 or more cigarettes daily), alcohol consumption (never, past, currently drinking 22.7 g or less alcohol daily, currently drinking 22.8 g or more alcohol daily), body mass index in $kg\,m^{-2}$ (18.4 or lower, 18.5–24.9, 25.0 or higher), education (up to 15 years of age, from 16 to 18 years, beyond the age of 19 years), family history of cancer (present, absent), time spent walking (less than 1 h daily, 1 h or longer daily) and meat consumption (45 g or less, 46–52 g, 53–62 g, 63 g or more daily). We repeated all analyses after excluding all subjects with colon and rectal cancer (45 with colon and 35 with rectal cancer) diagnosed within the first three years of follow-up, to eliminate the possibility that undiagnosed colorectal cancer that was present at baseline might influence the results for fruit and vegetable consumption. *P*-values to test for linear trends were estimated using the number of grams of fruit and vegetables consumed per day as a continuous variable. All *P*-values were two-tailed.

Results

Table 1 shows the characteristics of the subjects stratified by total fruit and vegetable consumption quartiles. No appreciable differences in median age were observed. Compared with those in the lowest quartile, the men in the highest quartile were more likely never to have smoked, to consume less alcohol, spend more time walking, have been educated to a high level and to consume more meat. We observed similar tendencies for women. Similar results were obtained when subject characteristics were analysed with respect to total vegetable and total fruit intakes (data not shown).

Table 2 presents the relative risk for colon cancer according to quartiles of total fruit and vegetable, total vegetable and total fruit consumptions. After adjustment for age and sex, consumption of total fruit and vegetables, total vegetables and total fruit showed no association with the risk of colon cancer. The age- and sex-adjusted RRs for

Table 1 Characteristics of subjects according to consumption of total fruit and vegetables in men and women

Characteristic	Quartile			
	1 (lowest)	2	3	4 (highest)
Energy-adjusted consumption (g)	≤543	544–616	617–697	≥698
Men				
Number of subjects	5130	5342	4786	4916
Age (years), mean ± SD	52.0 ± 7.6	51.1 ± 7.6	51.5 ± 7.7	52.2 ± 7.6
Current smoker (%)	65.3	62.3	57.3	54.4
Current drinker (%)	80.1	77.9	75.9	70.0
Body mass index ≥25.0 kg m^{-2} (%)	25.3	26.9	25.0	26.6
Walking time <1 h day^{-1} (%)	47.4	54.8	52.2	50.3
Family history of cancer in first-degree relative (%)	24.2	24.2	26.0	27.2
Education, in school until age 19 years or older (%)	8.0	13.5	15.0	16.6
Meat consumption per day ≥63 g (%)	14.1	24.8	30.9	35.6
Women				
Number of subjects	5332	5227	5619	5483
Age (years), mean ± SD	52.1 ± 7.5	52.1 ± 7.5	51.9 ± 7.4	52.5 ± 7.4
Current smoker (%)	8.3	6.7	5.7	5.9
Current drinker (%)	21.2	21.5	20.7	19.6
Body mass index ≥25.0 kg m^{-2} (%)	29.6	30.1	29.8	30.4
Walking time <1 h day^{-1} (%)	43.7	51.0	52.3	50.5
Family history of cancer in first-degree relative (%)	25.0	28.1	29.0	29.1
Education, in school until age 19 years or older (%)	7.9	11.0	13.7	14.3
Meat consumption per day ≥63 g (%)	15.8	22.9	25.8	24.8

SD – standard deviation.
Total fruit and vegetables consist of the following: orange, other fruits and fresh fruit juices, green leaf vegetables, carrots and pumpkins, tomatoes, cabbage and lettuce, Chinese cabbage and *tsukemono* (pickles).

Table 2 Relative risk (RR) (95% confidence interval) of colon cancer for fruit and vegetables consumption

Food group and variable	Quartile				*P*-value for trend
	1 (lowest)*	2	3	4 (highest)	
Total fruit and vegetables					
Energy-adjusted consumption (g)	≤543	544–616	617–697	≥698	
Number of cases	41	39	35	50	
Person-years of follow-up	76 960	77 650	76 570	76 495	
Age- and sex-adjusted RR	1.00	1.00 (0.65–1.56)	0.93 (0.59–1.47)	1.24 (0.82–1.88)	0.36
Multivariate RR1	1.00	0.92 (0.59–1.45)	0.83 (0.52–1.32)	1.13 (0.73–1.75)	0.62
Multivariate RR2	1.00	0.86 (0.51–1.45)	0.74 (0.43–1.28)	1.05 (0.64–1.75)	0.90
Total vegetables					
Energy-adjusted consumption (g)	≤245	246–277	278–312	≥313	
Number of cases	35	47	41	50	
Person-years of follow-up	77 878	77 269	75 271	77 257	
Age- and sex-adjusted RR	1.00	1.14 (0.74–1.84)	1.25 (0.79–1.95)	1.34 (0.87–2.07)	0.17
Multivariate RR1	1.00	1.10 (0.69–1.75)	1.13 (0.71–1.80)	1.24 (0.79–1.95)	0.34
Multivariate RR2	1.00	1.11 (0.65–1.88)	0.98 (0.57–1.70)	1.18 (0.70–1.98)	0.64
Total fruit					
Energy-adjusted consumption (g)	≤95	96–169	170–241	≥242	
Number of cases	47	47	35	36	
Person-years of follow-up	76 617	76 809	77 868	76 380	
Age- and sex-adjusted RR	1.00	1.33 (0.88–2.01)	1.18 (0.74–1.90)	1.52 (0.91–2.52)	0.17
Multivariate RR1	1.00	1.30 (0.85–1.98)	1.16 (0.71–1.89)	1.45 (0.85–2.47)	0.28
Multivariate RR2	1.00	1.55 (0.94–2.54)	1.41 (0.80–2.48)	1.43 (0.75–2.72)	0.31

RR1 – multivariate relative risk with all cases of colon cancer in analysis; RR2 – multivariate relative risk with cases diagnosed in the first three years of follow-up excluded from analysis.
Total fruit and vegetables consist of the following: orange, other fruits and fresh fruit juices, green leaf vegetables, carrots and pumpkins, tomatoes, cabbage and lettuce, Chinese cabbage and *tsukemono* (pickles).
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Total fruit consist of the following: orange, other fruits and fresh fruit juices.
Multivariate models included: sex; age (in years); smoking status (never, past, currently smoking 1–19 cigarettes daily, currently smoking over 20 cigarettes daily); alcohol consumption (never, past, currently drinking 22.7 g or less alcohol daily, currently drinking 22.8 g or more alcohol daily); body mass index in kg m^{-2} (18.4 or lower, 18.5–24.9, 25.0 or higher); education (up to 15 years of age, from 16 to 18 years, 19 years or older); family history of cancer (present, absent); walking time (less than 1 h daily, over 1 h daily); meat consumption (in quartiles – 45 g or less, 46–52 g, 53–62 g or 63 g or more daily).
* Referent category.