Int. J. Cancer: **118**, 991–997 (2006)

Associations between 5,10-methylenetetrahydrofolate reductase codon 677 and 1298 genetic polymorphisms and environmental factors with reference to susceptibility to colorectal cancer: A case-control study in an Indian population

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Although the incidence rate of colorectal cancer is very low, and rectal cancer remains more common in India, a significant increase in its incidence has been reported for both men and women over the last 2 decades. We evaluated *MTHFR* genetic susceptibility and common environmental risk factors in the development of colon and rectal cancer, and assessed the interactions between gene and environmental factors with colorectal cancer in a case-control study in the Indian population. The study included 59 colon cancer cases, 243 rectal cancer cases and 291 controls. The variant *MTHFR* 677T allele is rare, while the 1298C allele is common among Indians. *MTHFR* 677T showed no association with colon cancer (OR = 0.82; 95% CI 0.28–2.05) and a nonstatistically significantly elevated risk with rectal cancer (OR = 1.51; 95% CI 0.86–2.68), and *MTHFR* 1298 CC genotype was found to be associated with a significantly decreased risk for both colon cancer (OR = 0.30, 95% CI 0.09–0.81) and rectal cancer (OR = 0.43, 95% CI 0.23–0.80). High intake of nonfried vegetables or fruits was inversely associated with both colon and rectal cancer risk. Especially, the combination of a high intake of nonfried vegetables and *MTHFR* 1298CC genotype was associated with the lowest rectal cancer risk (OR = 0.22, 95% CI 0.09–0.52). Regarding alcohol consumption, indigenous Indian alcohol drinkers (OR = 2.26, 95% CI 0.86–6.36), and those consuming alcohol for duration more than 20 years (OR = 1.55, 95% CI 0.73–3.33), were at a somewhat higher rectal cancer risk. Moreover, the consumed alcohol amount (gram-years) may be also associated with colon or rectal cancer risk.
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Key words: 5,10-Methylenetetrahydrofolate reductase (MTHFR); polymorphisms; colorectal cancer; susceptibility

Colorectal cancer is a multifactorial disease involving genetic and environmental factors. Epidemiological studies have indicated that diets with a high intake of red meat and/or low consumption of vegetables, fruit and dietary fiber, obesity, high alcohol intake and smoking are associated with an increased colorectal cancer risk.[1–7] Folate is one of the important constituents of vegetables and fruit that may provide protection against colorectal cancer.[7] Folate is a water-soluble B vitamin that plays an essential role in many biochemical pathways such as DNA methylation and DNA synthesis. Its deficiency may lead to uracil misincorporation into DNA, DNA hypomethylation and inhibition of excision repair of DNA in human colon epithelial cells.[8–11] DNA methylation is a crucial epigenetic determinant in gene expression, maintenance of DNA integrity and stability, chromatin modifications and development of mutations.[12,13] Indeed, induction of DNA damage and disruption of its DNA integrity, impaired DNA repair and hypermutability are generally considered to be the primary mechanisms by which folate deficiency enhances colorectal carcinogenesis.[10,14–17]

5,10-Methylenetetrahydrofolate reductase (MTHFR) catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. The former converts dUMP to dTMP, a limiting nucleotide for DNA synthesis, whereas the latter is required to produce methionine for DNA methylation.[18] *MTHFR* variant genotypes may confer elevated plasma homocysteine levels, compared with the wild-type form consistent with a decline in remethylation of homocysteine to methionine.[19]

The *MTHFR* gene is polymorphic, with single nucleotide variants within codon 677 in exon 4 (C→T, ala to val) and codon 1298 in exon 7 (A→C, glu to ala). The codon 677 variant encodes a thermolabile enzyme with reduced activity that leads to reduced plasma folate levels.[20] In general, individuals with *MTHFR* 677 variant are at a relatively low colorectal cancer risk if they have low-risk diet (high folate and low alcohol). An explanation of how lower MTHFR activity could decrease the risk of colorectal cancer is that lessening dUMP-induced DNA damage would outweigh the negative effects of reduced DNA methylation in cases where folate intake is adequate. The second *MTHFR* variant, codon 1298 A to C, is associated to a much lesser degree with reduced enzymatic activity, and individuals carrying the variant have frequently normal homocysteine and plasma folate concentrations.[21,22] However, some studies have noted a significantly decreased risk of colon cancer with the *MTHFR* 1298CC genotype.[23,24]

It is well established that colorectal cancer is a leading cause of death in Western countries. In contrast to the developed world, however, the incidence rate of colorectal cancer is low in India, where rectal lesions are more common than tumors of the colon.[25] There is a 20-fold difference in the prevalence of colorectal cancer between the areas of highest and lowest incidences (North America and Australia *vs.* India).[26] The rural incidence rate for colorectal cancer in India is approximately half that of its urban population,[25] presumably reflecting a low consumption of meat and a high intake of dietary fiber, vegetables and fruit, and the presence of natural antioxidants such as curcumin in Indian cooking.

Although the incidence of colorectal cancer in Indians is low, a significant increase has been reported for both men and women over the last 2 decades, and migrant studies reveal a shift toward the rate prevalent in the host country,[27] This may be partly attributed to changes in dietary habits and lifestyle. In Indian populations, studies detecting associations between dietary factors, lifestyle and colorectal cancer have gained attention. However, little is known about *MTHFR* genetic polymorphisms on the suscepti-

Grant sponsor: Japanese Ministry of Education, Culture, Sports, Science and Technology; Grant sponsor: Japan Society for the Promotion of Science.
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Received 26 November 2004; Accepted after revision 20 June 2005
DOI 10.1002/ijc.21438
Published online 8 September 2005 in Wiley InterScience (www.interscience.wiley.com).

bility to colorectal cancer, and studies focusing on gene-environment interactions are limited. In order to identify *MTHFR* genetic susceptibility and common environmental risk factors, and to assess interactions between gene and environmental factors in terms of intake of vegetables, fruit and alcohol consumption with colorectal cancer, we conducted our case-control study.

Subjects and methods

Study participants

All subjects were recruited at the Cancer Institute, Chennai in South-Eastern India, from 1999 to 2001. Cases were first diagnosed as suffering from primary colorectal carcinoma and had been confirmed as competent to complete an interview. Controls were cancer-free healthy individuals selected from relatives/visitors to patients other than those with cancers in the gastrointestinal tract during the same period of case collection, and matched to cases for age and sex. A total of 435 cases between 17 and 88 years of age at the time of diagnosis, and 340 controls between 20 and 75 years of age agreed to the interview. Of these, 315 cases and 292 controls donated blood specimens for our study. Most of the study subjects were of Tamil and Telugu language groups, both of which are Dravidian in race, living in south India. Cases aged over 75 were excluded from the analysis, along with 2 cases and 1 control for which inexact data for food frequency were found, so that the final numbers were 302 cases (59 colon cancer patients and 243 rectal cancer patients) and 291 controls. Written informed consent was obtained from all study participants.

Data collection

Data were collected by trained interviewers at the Cancer Institute. Information was acquired on demographic variables, education, religion, mother tongue, marital status, socioeconomic conditions and family history of cancer using a standard questionnaire. Data on smoking status (including categories of tobacco, daily number smoked and duration of smoking habit), alcohol consumption (including categories of alcoholic beverages, frequencies and usual quantity of alcohol consumed) and chewing habits were also obtained simultaneously. Smokers were defined as persons who smoked a tobacco product at least once a day for at least 6 months. This definition was also applied to the chewing habit. Categories of tobacco included cigarette, bidi and chutta. Alcoholic beverages were classified into indigenous Indian varieties including toddy and arrack, and non-Indian beverages including beer, whisky and brandy. As a whole, alcoholic beverages were only consumed 1–2 times monthly, and quantity of alcohol consumed usually was less than 200 ml. As a result of low frequency and small quantity of alcohol consumed, alcohol drinkers were defined as individuals who drank at least once a month for more than 1 year for this special population. In addition, dietary information was collected using a food-frequency questionnaire (FFQ) specific to this population to measure long-term intake of food groups. Interviewers asked the subjects about the average intake frequency of food items per week over the past 1 year (for cases, 1 year before the diagnosis of colorectal cancer). Main categories of food groups were as follows: cereals and breads (n = 11 food items), beans (n = 6), vegetables (n = 22), meats (n = 4, including mutton, beef, pork and chicken), sea food (n = 7), eggs (n = 1), fruit (n = 12), pickles (n = 6), milk and dairy products (n = 10), beverages (n = 5), snacks and desserts (n = 18), spices (n = 7) and oil (n = 10). The categories of vegetables included green leaves, raw banana, ladies finger, drumstick, beans, karamani, cauliflower, tapiocca, potato, onion, carrot, beet root, radish, pumpkin, cucumber, brinjal, tomato, cabbage, yam, plantain stem, bitter guard and snake guard. The categories of fruits included lime, watermelon, guava, banana, orange, grape, mango, apple, papaya, jack, canned fruit and others. Food intake frequencies were classified into 6 categories: never (0), occasional or ≤1 time per month (0.2), 1 time per half month (0.5), 1 time per week (1), 2–4 times per week (3) and 5–7 times per week (6); values in parentheses are the weights assigned.

MTHFR genotyping

Genomic DNA was extracted from leukocytes of blood samples. *MTHFR* genotypes at C677T and A1298C sites were analyzed by PCR-based RFLP methods. The PCR primers for the C677T site were 5′-TGAAGGAGAAGGTGTCTGCGGGA-3′ and 5′-AGGACGGTGCGGTGAGAGTG-3′, which produce a 198 bp fragment. PCR products were digested by the restriction enzyme Hinf I to cut the product from the mutated allele into 175 and 23 bp fragments.[28] With respect to genotyping for the *MTHFR* A1298C polymorphism, 2 primers, 5′-GGGAGGAGCTGACCAGTGCAG-3′ and 5′-GGGGTCAGGCCAGGGGCAG-3′, were used to generate a 138 bp fragment, which was digested with Fnu4H I into 119 and 19 bp fragments.[18] To ensure reliability, a 20% random sample of cases and controls was genotyped twice by different researchers (J. Wang and J. Jiang), and the reproducibility confirmed to be 100%.

Statistical analysis

Differences in general characteristics between cases and controls were examined by using χ^2 test and t-test. For measuring associations between *MTHFR* genotypes or environmental exposure factors and colorectal cancer, ORs and 95% CIs were estimated from unconditional logistic regression models using the software package SAS (version 8.2) and adjusted for potential confounding factors such as age, sex, household income, education, religion, mother tongue, tobacco, alcohol, chewing habit and vegetarianism.

Statistical power calculations based on the prevalence of the 2 genetic polymorphisms and the sample size, our study has 70 and 95% power to detect the minimum odds ratio of 2.00 for *MTHFR* 677 and 1298 genotypes, respectively (α = 0.05, 2-sided test).

In order to calculate total smoked pack-years with different tobacco products, we calculated cigarette equivalents by assigning a weight of 1 for cigarettes, 0.25 for bidis and 0.5 for chuttas, based on grams of tobacco content. The amount (gram-years) of alcohol consumption was also calculated by finding the product of duration (years) and quantity (grams) of alcohol consumed monthly, the percentage of the alcohol was assumed as 3% for beer, 10% for toddy and 40% for the others.[29] Pack-years smoked and the amount of alcohol consumed were calculated to provide cumulative doses, and allow division into 2 groups for each (pack-years, ≤3 and >3; alcohol consumption, ≤800 and >800). Plans call for the detailed evaluation of the possible relations between dietary factors and colorectal carcinoma risk at some future point. Here, we only used the intake of total vegetables and fruit, which was the sum of assigned weights of various vegetables or fruit. On the whole, the intake of vegetables or fruit is frequent in Indian population, according to the sum of assigned weights, low intake or high intake groups of nonfried vegetables (≤21 and >21 servings per week) or fruit (≤3 and >3), and intake or nonintake groups of fried vegetables were stratified. The combined effects of *MTHFR* 677 and 1298 genotypes were calculated using individuals who were homozygous wild-type at both loci as the referent group. We also assessed the joint effects between genotypes and alcohol consumption using nondrinkers with wild-type for either *MTHFR* 677 or 1298 as the reference. Interactions between vegetables or fruit intake and *MTHFR* genotypes were also evaluated.

The distribution of *MTHFR* genotypes among controls was tested for Hardy-Weinberg equilibrium, and the distribution of *MTHFR* 677 and 1298 genotypes among cases and controls was estimated for haplotypes and linkage disequilibrium, using SNP Alyze (version 3.2) software.

Results

Selected characteristics of the study participants are presented in Table I. No significant differences were found between colon or rectal cancer cases and controls in terms of the distributions of sex, age, education level or religion. Both colon and rectal cancer

TABLE I – CHARACTERISTICS OF THE STUDY SUBJECTS

Variables	Controls (n = 291)	Cases			
		Colon cancer (n = 59)	p value[1]	Rectal cancer (n = 243)	p value[1]
Gender (male %)	182 (62.5)	40 (67.8)	0.45	157 (64.6)	0.62
Age groups					
≤40 yr	99 (34.0)	21 (35.6)		72 (29.6)	
>40 yr	192 (66.0)	38 (64.4)	0.30	171 (70.4)	0.03
Median (range)	50 (20–75)	50 (22–72)		50 (17–75)	
Mean (sd)	47.3 (12.6)	48.5 (12.0)	0.51	49.1 (14.1)	0.12
Current BMI (kg/m^2)[2]					
<25	221 (76.0)	51 (89.5)		205 (86.5)	
≥25	32 (11.0)	3 (5.3)		14 (5.9)	
≥27	38 (13.0)	3 (5.3)	0.08	18 (7.6)	0.01
Median (range)	21.7 (14.0–36.1)	19.5 (12.3–28.5)		20.0 (13.1–33.3)	
Mean (sd)	21.9 (4.3)	20.0 (3.8)	<0.01	20.5 (4.0)	<0.01
Education					
<Middle	88 (30.2)	18 (30.5)		85 (35.0)	
Middle and high	164 (56.4)	31 (52.5)		126 (51.9)	
>High	39 (13.4)	10 (17.0)	0.75	32 (13.2)	0.49
Religion					
Hindu	256 (88.0)	47 (79.7)		219 (90.1)	
Muslim	27 (9.3)	7 (11.9)		16 (6.6)	
Christian	8 (2.8)	5 (8.5)	0.08	8 (3.3)	0.50
Household income (rupees)					
<500	82 (28.2)	17 (28.8)		104 (42.8)	
500–1500	148 (50.9)	25 (42.4)		79 (32.5)	
>1500	61 (21.0)	17 (28.8)	0.35	60 (24.7)	<0.01
Mother tongue					
Tamil	185 (63.6)	27 (45.8)		127 (52.3)	
Telugu	77 (26.5)	20 (33.9)		90 (37.0)	
Urdu	9 (3.1)	3 (5.1)		7 (2.9)	
Other	20 (6.9)	9 (15.3)	0.04	19 (7.8)	0.05
Family history					
None	174 (59.8)	47 (79.7)		226 (93.0)	
Colorectal cancers	0 (0.0)	3 (5.1)		1 (0.4)	
Other cancers	117 (40.2)	9 (15.3)	<0.01	16 (6.6)	<0.01

[1]By chi-square test or t test.–[2]Data missing for 2 subjects with colon cancer and 6 subjects with rectal cancer.

cases had lower current BMI than controls. In respect to household income, a lower annual income (<500 rupees) was more often found among rectal cancer cases. However, controls had a higher frequency of family history of other cancers than cases, because controls were selected from relatives/visitors to the patients having nongastrointestinal cancers. There was a slight difference in the distribution of mother tongue between cases and controls. Marital status and types of residence were also compared between cases and controls, but there were no differences (data not shown).

Data for smoking status, drinking status, chewing habit and vegetarians and risks to colon and rectal cancer are shown in Table II. Nonsmokers and nondrinkers were more common in both cases and controls. For categories of tobacco products, cigarette smoking was more frequent among colon cancer cases (18.6%) and less among rectal cancer cases (11.1%) compared to controls (14.4%). Both bidi and chutta smokers exhibited an increased rectal cancer risk (bidi: OR = 1.44, 95% CI 0.71–2.94; chutta: OR = 4.47, 95% CI 1.12–23.9) but without statistical significance for bidi and with a wide 95% confidence interval for chutta because of the small numbers. Although total pack-years were stratified into 2 groups, no significant risk derived from pack-years was found.

With respect to alcohol, no significant differences were found between colon or rectal cancer cases and controls in distribution of non-Indian-alcohol drinkers (p = 0.72; 0.71, respectively), but Indian-alcohol drinkers may be at a somewhat higher rectal cancer risk (OR = 2.26, 95% CI 0.86–6.36). According to stratification by drinking duration, alcohol consumption for more than 20 years was associated with the tendency for an increased risk of rectal cancer (OR = 1.55, 95% CI 0.73–3.33). Regarding the amount of consumed alcohol, for both colon and rectal cancers, the group with less than eight-hundred gram-years showed weakly decreased risk (colon: OR = 0.77, 95% CI 0.21–2.21; rectal: OR = 0.66, 95% CI 0.32–1.35), and that with more than 800 gram-years showed a slightly elevated risk (colon: OR = 1.53, 95% CI 0.55–3.86; rectal: OR = 1.56, 95% CI 0.82–3.02).

The distribution of betel chewing showed no statistical differences between cases and controls. A decreased colon or rectal cancer risk was found for chewing habits but had not reached statistical significance. However, a significantly increased rectal cancer risk was found for vegetarianism.

The frequencies of *MTHFR* genotypes and the association between genotypes and cancers are summarized in Table III. The allele frequency for *MTHFR* 677T was 0.05 among colon cancer cases and 0.08 among rectal cancer cases, compared with 0.06 among controls. The *MTHFR* 677TT genotype in the Indian population is extremely rare, absent among colon cancer cases and controls, and was present in only 2 rectal cancer cases. The observed frequencies of MTHFR 677 genotypes among controls (CC, 87.6%; CT, 12.4%) were in accordance with the Hardy-Weinberg equilibrium (p = 0.26). The *MTHFR* 677T allele was found no association with colon cancer (OR = 0.82, 95% CI 0.28–2.05) and a nonstatistically significantly elevated risk with rectal cancer (OR = 1.51, 95% CI 0.86–2.68). The allele frequencies for *MTHFR* 1298C were 0.27, 0.33 and 0.41 in the colon and rectal cancer groups and controls, respectively. The distribution of *MTHFR* 1298 genotypes among controls (AA, 36.1%; AC, 46.4%; and CC, 17.5%) also agreed with that expected from the Hardy-Weinberg equilibrium (p = 0.54), which was significantly different from colon cancer cases (AA, 54.2%; AC, 37.3%; and CC, 8.5%; p = 0.02) and rectal cancer cases (AA, 44.9%; AC, 44.4% and CC, 10.7%; p = 0.03). As compared with their counterparts with the *MTHFR* 1298 AA genotype, subjects carrying the *MTHFR* 1298

TABLE II – DISTRIBUTION OF SMOKING, DRINKING, CHEWING AND VEGETARIANS AND ORS FOR COLON AND RECTAL CANCER

Habit	Controls (n = 291)	Colon cancer (n = 59)	OR (95% CI)	Rectal cancer (n = 243)	OR (95% CI)
Smoking status[1]					
Cigarette					
Never	249 (85.6)	48 (81.4)	1.00 (Ref)	216 (88.9)	1.00 (Ref)
Smokers	42 (14.4)	11 (18.6)	1.30 (0.54–2.96)	27 (11.1)	0.63 (0.34–1.15)
Bidi					
Never	266 (91.4)	54 (91.5)	1.00 (Ref)	218 (89.7)	1.00 (Ref)
Smokers	25 (8.6)	5 (8.5)	1.12 (0.34–3.20)	25 (10.3)	1.44 (0.71–2.94)
Chutta					
Never	288 (99.0)	58 (98.3)	1.00 (Ref)	232 (95.5)	1.00 (Ref)
Smokers	3 (1.0)	1 (1.7)	1.63 (0.07–16.84)	11 (4.5)	4.47 (1.12–23.95)
Pack-years[2]					
0	225 (77.3)	44 (74.6)	1.00 (Ref)	188 (77.4)	1.00 (Ref)
≤3	33 (11.3)	8 (13.6)	1.38 (0.51–3.49)	19 (7.8)	0.72 (0.35–1.44)
>3	33 (11.3)	7 (11.9)	1.07 (0.37–2.85)	36 (14.8)	1.26 (0.67–2.39)
Drinking status[3]					
Non-Indian alcohol					
Never	247 (84.9)	49 (83.0)	1.00 (Ref)	209 (86.0)	1.00 (Ref)
Drinkers	44 (15.1)	10 (17.0)	1.25 (0.53–2.72)	34 (14.0)	1.02 (0.59–1.77)
Indian alcohol					
Never	282 (96.9)	57 (96.6)	1.00 (Ref)	227 (93.4)	1.00 (Ref)
Drinkers	9 (3.1)	2 (3.4)	1.22 (0.18–5.35)	16 (6.6)	2.26 (0.86–6.36)
All alcohol					
Never	238 (81.8)	48 (81.4)	1.00 (Ref)	198 (81.5)	1.00 (Ref)
Drinkers	53 (18.2)	11 (18.6)	1.13 (0.50–2.38)	45 (18.5)	1.08 (0.66–1.79)
Duration (years)[4]					
<20	35 (12.0)	8 (13.6)	1.19 (0.46–2.79)	25 (10.3)	0.83 (0.44–1.53)
≥20	18 (6.2)	3 (5.1)	0.99 (0.22–3.31)	20 (8.2)	1.55 (0.73–3.33)
Amount (gram-years)[4]					
≤800	29 (10.0)	4 (6.8)	0.77 (0.21–2.21)	16 (6.6)	0.66 (0.32–1.35)
>800	24 (8.2)	7 (11.9)	1.53 (0.55–3.86)	29 (11.9)	1.56 (0.82–3.02)
Chewing habit[5]					
No	236 (81.1)	50 (84.7)	1.00 (Ref)	202 (83.1)	1.00 (Ref)
Yes	55 (18.9)	9 (15.3)	0.61 (0.25–1.34)	41 (16.9)	0.78 (0.47–1.30)
Vegetarianism[6]					
No	258 (88.7)	49 (83.0)	1.00 (Ref)	195 (80.2)	1.00 (Ref)
Yes	33 (11.3)	10 (17.0)	1.87 (0.77–4.29)	48 (19.8)	1.83 (1.04–3.26)

[1]Adjusted for gender, age, household income, education, religion, mother tongue, drinking, chewing and vegetarianism.–[2]Pack-years calculated by different tobacco products (weight of 1 for cigarettes, 0.25 for bidis and 0.5 for chuttas).–[3]Adjusted for gender, age, household income, education, religion, mother tongue, smoking, chewing and vegetarianism.–[4]Never drinkers of all alcohol as the referent group.–[5]Adjusted for gender, age, household income, education, religion, mother tongue, smoking, drinking and vegetarianism.–[6]Adjusted for gender, age, household income, education, religion, mother tongue, smoking, drinking and chewing.

TABLE III – GENOTYPE FREQUENCIES, AND ADJUSTED ORS(95% CIS)[1] FOR COLON, RECTAL AND COLORECTAL CANCERS WITH POLYMORPHISMS OF MTHFR 677 AND 1298

Genotype	Control subjects (n = 291) n (%)	Colon cancer (n = 59) n (%)	OR (95% CI)	Rectal cancer (n = 243) n (%)	OR (95% CI)	Colorectal cancers (n = 302) n (%)	OR (95% CI)
MTHFR 677							
CC	255 (87.6)	53 (89.8)	1.00 (Ref)	204 (84.0)	1.00 (Ref)	257 (85.1)	1.00 (Ref)
CT	36 (12.4)	6 (10.2)	0.82 (0.28–2.05)	37 (15.2)	1.40 (0.79–2.49)	43 (14.2)	1.22 (0.72–2.09)
TT	0 (0.00)	0 (0.00)	NA	2 (0.8)	NA	2 (0.7)	NA
CT or TT	36 (12.4)	6 (10.2)	0.82 (0.28–2.05)	39 (16.0)	1.51 (0.86–2.68)	45 (14.9)	1.31 (0.78–2.23)
MTHFR 1298							
AA	105 (36.1)	32 (54.2)	1.00 (Ref)	109 (44.9)	1.00 (Ref)	141 (46.7)	1.00 (Ref)
AC	135 (46.4)	22 (37.3)	0.43 (0.22–0.82)	108 (44.4)	0.70 (0.45–1.06)	130 (43.0)	0.62 (0.42–0.92)
CC	51 (17.5)	5 (8.5)	0.30 (0.09–0.81)	26 (10.7)	0.43 (0.23–0.80)	31 (10.3)	0.40 (0.22–0.70)
AC or CC	186 (63.9)	27 (45.8)	0.40 (0.22–0.74)	134 (54.1)	0.62 (0.42–0.93)	161 (53.3)	0.56 (0.38–0.81)
Combined genotypes							
CC and AA	83 (28.5)	28 (47.4)	1.00 (Ref)	83 (34.2)	1.00 (Ref)	111 (36.8)	1.00 (Ref)
CC and AC	121 (41.6)	21 (35.6)	0.42 (0.21–0.83)	95 (39.1)	0.69 (0.43–1.11)	116 (38.4)	0.61 (0.39–0.93)
CC and CC	51 (17.5)	4 (6.8)	0.22 (0.06–0.64)	26 (10.7)	0.45 (0.23–0.86)	30 (9.9)	0.39 (0.21–0.70)
CT or TT and AA	22 (7.6)	4 (6.8)	0.54 (0.13–1.74)	26 (10.7)	1.17 (0.56–2.51)	30 (9.9)	0.99 (0.49–2.00)
CT or TT and AC	14 (4.8)	1 (1.7)	0.16 (0.01–0.98)	13 (5.3)	0.97 (0.38–2.48)	14 (4.6)	0.71 (0.29–1.73)
CT or TT and CC	0 (0.0)	1 (1.7)	NA	0 (0.0)	NA	1 (0.3)	NA

[1]Adjusted for gender, age, household income, education, religion, mother tongue, smoking, drinking, chewing and vegetarianism.

AC genotype were at a low risk for either colon cancer (OR = 0.43, 95% CI 0.22–0.82) or possibly rectal cancer (OR = 0.70, 95% CI 0.45–1.06). Moreover, individuals carrying the *MTHFR* 1298CC genotype showed a significantly decreased risk for both colon cancer (OR = 0.30, 95% CI 0.09–0.81) and rectal cancer (OR = 0.43, 95% CI 0.23–0.80). The p values for trend tests of the MTHFR 1298 genotypes were 0.005 (χ^2 = 7.93) for colon cancer and 0.007 (χ^2 = 7.41) for rectal cancer.

The distribution of cases and controls for *MTHFR* polymorphisms is in line with C677T and A1298C being in complete linkage disequilibrium (p = 0.000, χ^2 test). Estimation of *MTHFR* haplotype frequencies for combinations of C677T and A1298C alleles also demonstrated the following statistically significant case-control differences (p = 0.004): 0.61 677C/1298A; 0.07 677T/1298A; 0.31 677C/1298C; and 0.004 677T/1298C among cases and 0.53 677C/1298A; 0.06 677T/1298A; 0.40 677C/1298C; and 0.000 677T/1298C among controls.

Combined effects of the *MTHFR* 677 and 1298 genotypes on risk of colon, rectal and colorectal cancer were also analyzed (see Table III). No subject in our study carried homozygous mutant alleles at both sites (677TT/1298CC). Only 1 case carried the 677CT/1298CC genotype, and individuals who carried 677CT(TT)/1298AC were rare. When *MTHFR* 1298AA genotype was only considered, *MTHFR* 677T showed an inverse association with colon cancer risk (OR = 0.54, 95% CI 0.13–1.74), and combined 677CT/1298AC genotypes appeared a decreased risk for colon cancer compared with the homozygous wild-type 677CC/1298AA (OR = 0.16, 95% CI 0.01–0.98), but these results need to be confirmed because of small numbers.

Interactions for alcohol, vegetable intake and *MTHFR* polymorphisms are presented in Table IV. For alcohol consumption, no significant link was found between the *MTHFR* 677 polymorphism and rectal cancer. A nonstatistically significant association was observed among drinkers with the *MTHFR* 1298AA genotype for rectal cancer (OR = 1.97, 95% CI 0.88–4.57). With regard to vegetable intake, nonfried and fried categories were individually analyzed for their effects. With high intake of nonfried vegetables, a clearly decreased risk was found for both colon cancer (adjusted OR = 0.40; 95% CI, 0.20–0.84) and rectal cancer (adjusted OR = 0.47; 95% CI, 0.28–0.75), comparing to the low intake group. However, with fried vegetables, the lower risk was observed with low consumption for both colon cancer (adjusted OR = 0.78; 95% CI, 0.40–1.46) and rectal cancer (adjusted OR = 0.62; 95% CI, 0.40–0.96). For rectal cancer with the *MTHFR* 677T allele, there appeared to be risk reduction among those with high intake of nonfried vegetables (OR = 0.66, 95% CI 0.30–1.42). The lowest risk for rectal cancer (OR = 0.22, 95% CI 0.09–0.52) was found among the high intake group of nonfried vegetables with the *MTHFR* 1298CC genotype. Similarly, interactions of fried vegetable intake with *MTHFR* genotypes were apparent, but not as strong as in the nonfried case.

In addition, high intake of fruit also was associated with a somewhat reduced risk for both colon cancer (adjusted OR = 0.65; 95% CI, 0.35–1.23) and rectal cancer (adjusted OR = 0.75; 95% CI, 0.50–1.13). There was no significant interaction with *MTHFR* genetic polymorphisms regarding susceptibility to colon or rectal cancer.

TABLE IV – RELATIONSHIP OF ALCOHOL AND VEGETABLE INTAKE TO RECTAL AND COLORECTAL CANCER RISK STRATIFIED BY MTHFR GENOTYPE

	MTHFR677 genotype				MTHFR1298 genotype					
	CC[1]	OR (95% CI)[2]	CT or TT[1]	OR (95% CI)[2]	AA[1]	OR (95% CI)[2]	AC[1]	OR (95% CI)[2]	CC[1]	OR (95% CI)[2]
Alcohol										
Never drinker										
Rectal cancer	166/208	1.00 (Ref)	32/30	1.53 (0.82–2.86)	85/91	1.00 (Ref)	93/105	0.88 (0.55–1.41)	20/42	0.44 (0.22–0.86)
Colorectal cancer	208/208	1.00 (Ref)	38/30	1.38 (0.77–2.47)	110/91	1.00 (Ref)	111/105	0.75 (0.48–1.56)	25/42	0.42 (0.22–0.78)
Drinker										
Rectal cancer	38/47	1.10 (0.64–1.88)	7/6	1.46 (0.41–5.42)	24/14	1.97 (0.88–4.57)	15/30	0.47 (0.21–0.99)	6/9	0.72 (0.20–2.49)
Colorectal cancer	49/47	1.05 (0.64–1.73)	7/6	1.09 (0.31–3.89)	31/14	1.69 (0.79–3.66)	19/30	0.45 (0.22–0.90)	6/9	0.51 (0.15–1.70)
Non-fried vegetables[3]										
Low intake[4]										
Rectal cancer	58/40	1.00 (Ref)	14/4	1.97 (0.60–7.78)	33/19	1.00 (Ref)	33/17	0.68 (0.27–1.72)	6/8	0.39 (0.09–1.59)
Colorectal cancer	73/40	1.00 (Ref)	15/4	1.84 (0.58–7.09)	45/19	1.00 (Ref)	36/17	0.57 (0.24–1.39)	7/8	0.37 (0.10–1.38)
High intake[4]										
Rectal cancer	146/215	0.50 (0.29–0.84)	25/32	0.66 (0.30–1.42)	76/86	0.46 (0.21–0.95)	75/118	0.33 (0.15–0.67)	20/43	0.22 (0.09–0.52)
Colorectal cancer	184/215	0.49 (0.30–0.80)	30/32	0.58 (0.28–1.17)	96/86	0.45 (0.22–0.88)	94/118	0.30 (0.15–0.58)	24/43	0.19 (0.08–0.43)
Fried vegetables										
Intake										
Rectal cancer	138/158	1.00 (Ref)	29/24	1.63 (0.83–3.24)	78/63	1.00 (Ref)	71/84	0.58 (0.34–0.99)	18/35	0.39 (0.18–0.82)
Colorectal cancer	171/158	1.00 (Ref)	31/24	1.27 (0.67–2.44)	101/63	1.00 (Ref)	83/84	0.54 (0.33–0.88)	23/35	0.40 (0.20–0.79)
Non-intake										
Rectal cancer	66/97	0.66 (0.41–1.04)	10/12	0.73 (0.26–2.05)	31/42	0.45 (0.23–0.90)	37/51	0.46 (0.24–0.86)	8/16	0.24 (0.08–0.67)
Colorectal cancer	81/97	0.70 (0.46–1.06)	14/12	0.96 (0.39–2.40)	40/42	0.59 (0.31–1.09)	47/51	0.48 (0.27–0.85)	8/16	0.21 (0.07–0.56)

[1]Numbers of cases/controls.–[2]Adjusted for gender, age, household income, education, religion, mother tongue, smoking, drinking and chewing.–[3]These vegetables as the predominant source of folate in Indian population.–[4]Based on a sum of assigned weights of various vegetables (low intake group ≤21, high intake group>21).

Discussion

Several epidemiological studies have focused on associations between *MTHFR* polymorphisms and colon cancer in Caucasians.[23,30–33] Two demonstrated an inverse association between *MTHFR* 677TT genotype and colorectal cancer when either folate intake was high or alcohol consumption was low, and a positive association with low folate intake or high alcohol intake.[30,31] Furthermore, 2 studies revealed weak inverse associations between *MTHFR* 677TT genotype and colon cancer independent of intake of folate or alcohol.[32,33] One study found no association between the low activity *MTHFR* 677TT genotype and colon cancer,

and no relations with the use of alcohol, but a weak positive association was shown with low folate intake (<400 μg/day). Moreover, a significant inverse association was demonstrated between *MTHFR* 1298CC genotype and colon cancer.[23] In addition, Curtin *et al.* recently reported a strong inverse association between colon cancer and *MTHFR* 1298CC genotype among women in a largely Caucasian population.[24]

The participants in our study were mainly of Tamil and Telugu language groups. Although slight differences in the distribution were present between cases and controls, both belonging to the same Dravidian race, and the distribution of *MTHFR* genotypes demonstrated no significant variation between Tamil and Telugu participants. We also adjusted for mother tongue in our analysis.

Our results showed that *MTHFR* 677T allele was extremely rare (0.06) in healthy Dravidian Indians, in accordance with the low prevalence of 677 mutation reported in Asian Indians and Tamilians,[34,35] differing from the case with Whites (0.30–0.35)[23,30,31] and other Asian peoples (0.41–0.44).[36,37] In contrast, the frequency of the *MTHFR* 1298C allele (0.41) is higher than in either,[23,36,37] which is also similar to that reported among Tamilians.[35] To date, associations of *MTHFR* genetic polymorphisms with coronary artery diseases have been evaluated in Indians.[34,38]

The *MTHFR* 677T allele was found no association with colon cancer [OR = 0.82 (0.28–2.05)] in our study, similar to the earlier studies,[23,24] albeit not as strong as the reports in the meta-analysis undertaken by Houlston *et al.* [OR = 0.77 (0.62–0.92)].[39] Furthermore, an indication of an increased rectal cancer risk with the *MTHFR* 677T allele was also found [OR = 1.51 (0.86–2.68)]. The inconsistent results in our study may be due to the rare 677T allele in Indians and result in our sample size was insufficient to evaluate the association of *MTHTR* 677 genotypes with colon or rectal cancer.

In agreement with the findings of Keku *et al.*[23] and Curtin *et al.*,[24] our study demonstrated strong inverse associations between *MTHFR* 1298CC genotype and colon cancer [OR = 0.30 (0.09–0.81)] or rectal cancer [OR = 0.43 (0.23–0.80)], and confirmed that *MTHFR* 1298 may be more important than 677 genotypes for colorectal cancer risk. Because the location of 677 (NH_2-terminal) and 1298 (COOH-terminal) is distinct, and the amino acid affected by 1298 single nucleotide substitution (A→C, glu to ala) is located near the binding site for the allosteric MTHFR inhibitor S-adenosyl-methionine, may possibly affect feedback inhibition. In addition, the balance of DNA synthesis and DNA methylation determined by *MTHFR* polymorphisms may play an important role in the regulation of gene expression influencing cancer risk. It has been suggested that relationships between *MTHFR* polymorphisms and colorectal cancer may be different by gender and age distribution, we also detected the associations between *MTHFR* 1298 genotypes and rectal cancer risk by gender and age groups, but no significant differences were found.

The haplotype frequencies of *MTHFR* 677 and 1298 were also estimated, and significant case-control differences were found. However, we have not examined the association with cancer risk because the sample size was small and haplotypes might be unreliable.

We evaluated the associations of smoking status and colon or rectal cancer risk. Although specific categories of tobacco, bidi and chutta exhibited an increased rectal cancer risk, for bidi no statistical significance was found and for chutta with a wide confidence interval. Furthermore, total pack-years of 3 tobacco categories were not found to be associated with rectal cancer risk. For alcohol consumption, indigenous Indian alcohol drinkers may be at a somewhat higher rectal cancer risk [OR = 2.26 (0.86–6.36)], and drinking duration for more than 20 years was found to be an increased risk tendency for rectal cancer [OR = 1.55 (0.73–3.33)]. Furthermore, in order to detect the association between cumulative doses of alcohol consumption and colon or rectal cancer risk for light drinkers, we made an attempt to calculate the amount (gram-years) and found that the amount for less than 800 gram-years was associated with a somewhat lower colon or rectal cancer risk [colon: OR = 0.77 (0.21–2.21); rectal: OR = 0.66 (0.32–1.35)] and that with over eight-hundred gram-years was associated with a somewhat higher colon or rectal cancer risk [colon: OR = 1.53 (0.55–3.86); rectal: OR = 1.56 (0.82–3.02)]. In our study, although the definition of alcohol drinkers (who drink at least once a month for more than 1 year) may be too inclusive, if drinkers were defined as usual (who drink at least once a week or a day), then there were not drinkers in our study. In addition, there may be underreporting of alcohol intake relating to religion, which should be taken into account. We also assessed the interaction of alcohol consumption and *MTHFR* polymorphisms with susceptibility to rectal cancer, and found that drinkers with *MTHFR* 1298AA genotype were related to an increased risk tendency for rectal cancer [OR = 1.97 (0.88–4.57)]. As introduced above, *MTHFR* 1298 wild-type (AA) with high enzyme activity may promote the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, producing a low 5,10-methylenetetrahydrofolate pool level that leads to misincorporation of uracil for thymidine during DNA synthesis. Moreover, some investigators have hypothesized that colorectal cancer risk associated with alcohol was related to its anti-folate effect[40] or more specifically to its effects on DNA methylation,[41] These may explain the elevated risk among drinkers carrying the high activity *MTHFR* genotype.

High intake of nonfried vegetables or fruit showed inverse association with both colon and rectal cancer in our study, and these vegetables and fruit are thought to be the predominant source of dietary folate intake in Indian population. Especially, the combination of high intake of non-fried vegetables and *MTHFR* 1298CC genotype demonstrated the lowest risk for rectal cancer [OR = 0.22 (0.09–0.52)].

In conclusion, this case-control study exhibited that the frequency of *MTHFR* 677T allele is rare, while *MTHFR* 1298C allele is common among Indians, and *MTHFR* 1298CC genotype was significantly associated with decreased colon and rectal cancer risk. Furthermore, our study confirmed the suggestion that *MTHFR* 1298 polymorphism may be more important than *MTHFR* 677 polymorphism for colorectal cancer. The intake of vegetables is frequent on the whole in Indian population, and the high intake of nonfried vegetables clearly showed a reduced risk for both colon and rectal cancers. Furthermore, the combination of high intake of nonfried vegetables and *MTHFR* 1298CC genotype was found to be associated with the lowest rectal cancer risk. These may explain why the incidence rate of colorectal cancer is very low in Indian populations, taken together with high level of physical activity and walking, high intake of dietary folate from vegetables and fruit but very limited alcohol consumption. For the light drinkers, long-term alcohol consumption and going beyond a certain cumulative amount also showed an increased risk trend for rectal cancer. However, the low prevalence of colorectal cancer in India may be associated with other dietary factors such as high curry intake and low red meat intake as well as the other genetic variations in metabolic enzymes and DNA repair enzymes, which remain to be confirmed.

Acknowledgements

The authors are grateful to Dr. V. Shanta and Dr. T. Rajkumar (Cancer Institute, Chennai, India) for their support and cooperation, and Dr. M. A. Moore for linguistic advice. Jingwen Wang was also financially supported by the Japan Society for the Promotion of Science and its postdoctoral fellowship for researchers from abroad.

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■解　説

家族性大腸腺腫症の化学予防

石川秀樹*,**

家族性大腸腺腫症に対する大腸癌の化学予防について，非ステロイド系抗炎症剤（NSAIDs）の一つであるスリンダクを用いた臨床試験が多く行われている．スリンダクは間違いなく大腸ポリープを縮小させるが，重篤な副作用の発生率が高いこと，ポリープが縮小しても進行癌が発生した症例があることより，まだ実地医療で使用することはできないと考える．消化管傷害の少ないCOX2選択的阻害剤も心疾患の副作用が指摘され，予防薬としての使用は困難である．NSAIDs以外にもビタミンや食品成分を用いた臨床試験が行われている．これらは副作用は少ないが，効果は不十分であり，さらなる研究が必要である．

I.　はじめに

家族性大腸腺腫症における大腸発癌の予防は，大腸を予防的に摘除することが一般的である．しかし，大腸摘除後の下痢などにより患者の生活の質（QOL）を低下させるため，摘除せずに発癌を予防することが望ましい．したがって，発癌を予防するための研究が積極的に行われているが，実用化された発癌予防法はまだない．

家族性大腸腺腫症における大腸癌は，一般集団に比べ発癌率が高いため，比較的，少人数，短期間で発癌予防に関する臨床試験の結果が得られること，多数存在する大腸腺腫を発癌の中間代理指標とすることができること，分子生物学的知見から家族性大腸腺腫症で得られた情報が散発性大腸癌にも当てはめることができることが明らかになったことなどから，家族性大腸腺腫症を対象とした発癌予防の臨床試験が積極的に行われるようになった．

II.　非ステロイド系抗炎症剤による大腸発癌予防

予防候補物質でもっとも多く報告されているのは，非ステロイド系抗炎症剤（NSAIDs）のsulindac（スリンダク）である．NSAIDsの一つであるindomethacin（インドメサシン）が化学発癌によるラット大腸癌の発生を予防することを1980年にKudoら[1]が最初に見いだして以来，ラットやマウスを用いた実験で各種のNSAIDsが大腸発癌を予防することがいくつも報告されている．また，人においても，アスピリンの長期服用者に大腸癌の罹患が少ないことが，症例対照研究[2]やコホート研究[3]で報告されている．これらより，アスピリンを含むNSAIDsが大腸癌を予防する可能性があること，スリンダクはプロドラッグであり，その他のNSAIDsに比べて消化管への傷害が少ないことより，スリンダクを用いた家族性大腸腺腫症に対する発癌予防試験が行われるようになった．1983年にWaddellら[4]が家族性大腸腺腫症患者4例に対してスリンダクを投与し，直腸のポリープがほとんど消失したことを報告したのが最初である．その後，多数の臨床試験が行われ，それらの報告のすべてで，スリンダクは家族性大腸腺腫症のポリープを退縮させると報告されている．その機序として，スリンダクがアポトーシスを亢進させることが考えられている．

しかし，スリンダクやその他のNSAIDsの投与により，消化管の潰瘍や穿孔などの重篤な副作用が報告されている[5-7]．

本来，NSAIDsは慢性リウマチなどによる疼痛に対する痛み止めとして用いられるものであり，長期間，連続して大量に使用するものではない．短期間では副作用が出なくても1年以上投与後に突然，胃穿孔などの重篤な副作用が発生することもあるため，長期間の投与には厳重な注意が必要である．

さらに，スリンダクを投与してポリープは退縮したにもかかわらず，進行癌が発生したとの症例が複数報告されている[8-10]．また，散発性の大腸腺腫患者においても，スリンダクの投与中に直腸癌が発生したことも報告されている[11]．本当にNSAIDsが大腸癌を予防するかどうかは，長期間の追跡調査などにより慎重に検討する必要がある．

正常の粘膜では発現していないcyclooxygenase-2（COX2）が大腸癌組織では発現していることから，COX2が大腸発癌に関与していると考えられること，正常の消化管粘膜にはCOX2は発現していないためCOX2を阻害しても消化管粘膜に傷害を与える可能性が低いことなどから，最近になり，celecoxib（セレコキシブ）やrofecoxib（ロフェコキシブ）などのCOX2選択的阻害剤による臨床試験[12-14]が行われ，米国では，家族性大腸腺腫症に対するポリープの縮小を目的としたセレコキシブ投与が承認さ

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家族性腫瘍 第6巻 第1号（2006年）p.33-35

れていた．しかし，2005年になり，セレコキシブ[15]やロフェコキシブ[16]による大規模な大腸腺腫発生予防試験において，COX2選択的阻害剤の長期投与により心血管イベントが2倍程度上昇することがわかり，これらのCOX-2選択的阻害剤の使用は困難となっている．

スリンダク以外のNSAIDsではインドメサシンの坐剤[17]や経口投与[18]による臨床試験が行われている．インドメサシンでも大腸ポリープは減少するが，消化管粘膜傷害による貧血も報告されており，長期間の継続した投与には問題が多いと考える．

III. その他の物質による大腸発癌予防

抗癌剤の一つである5-fluorouracilの坐剤の投与でも大腸ポリープの減少が報告されているが，長期投与には副作用の問題が大きいと思われる．他には，ビタミンC，ビタミンE，カルシウム，小麦ふすま，docosahexaenoic acid (DHA)，緑茶抽出物などのビタミン剤や食品成分を用いた報告がある．これらはNSAIDsに比べて副作用は少ないが，スリンダク程度の有効性が認められたものはない．著者らは緑茶抽出物を用いた二重盲検試験（J-FAPP Study）を現在実施中[19]であり，2006年にはその効果が明らかになる予定である．

また，家族性大腸腺腫症の同一家系内でも，発癌時期やポリープの大きさに差が見られることより，大腸腫瘍の増大や癌化には，遺伝子変異だけではなく，運動や食事，喫煙などの環境要因も関与していることが考えられる．家族性大腸腺腫症の環境要因の研究はこれまでほとんど行われていなかったが，食事調査などの研究をこれからは積極的に行うべきであろう．

今後，これらの新しい薬の開発や生活指導により，家族性大腸腺腫症患者の大腸切除の手術時期を遅らせることや，手術を回避できるようになることが望まれる．

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Chemoprevention of Carcinogenesis in Familial Adenomatous Polyposis

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A large number of clinical trials have been performed using sulindac, a non-steroidal anti-inflammatory drug (NSAID). Sulindac reduces the size and number of large bowel polyps. However, it cannot be used for this indication in clinical settings as yet, because of the frequent occurrence of serious gastrointestinal side effects. There are a number of cases in which aggressive tumors developed despite a reduction in the size of polyps. In addition to NSAIDs, clinical trials have been performed using vitamins and dietary components. These show minimal side effects, but their efficacy is still insufficient for clinical use, and further studies are anticipated.
(J Fam Tumor 2006 ; 6 : 33−35)

American Journal of Gastroenterology

Published by Blackwell Publishing

ISSN 0002-9270
doi: 10.1111/j.1572-0241.2006.00308.x

A Novel Germline Mutation of *MSH2* in a Hereditary Nonpolyposis Colorectal Cancer Patient with Liposarcoma

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BACKGROUND: One of the clinical features of hereditary nonpolyposis colorectal cancer (HNPCC) is a high incidence of multiple primary neoplasms arising in various organs including the gastrointestinal and genitourinary tracts. Among extracolonic tumors, a limited number of soft tissue sarcomas associated with HNPCC have been reported, and the mechanism underlying liposarcoma in HNPCC patients remains unclear.

AIM: We herein report the case of a HNPCC patient with liposarcoma, with the goal of elucidating the involvement of a mismatch repair deficiency in the tumor.

METHODS AND RESULTS: A 40-yr-old Japanese patient, who had a past history of adenocarcinoma of the rectum and transitional cell carcinoma of the urinary bladder, developed a liposarcoma in his left thigh. Although his family history did not fulfill the revised Amsterdam criteria, his blood sample was subjected to genetic testing. Direct sequencing of the genomic DNA from the blood identified an AT deletion at codon 677 in exon 13 of *hMSH2*, a pathogenic mutation that has not been reported before. The expression of MSH2 in the liposarcoma and rectal cancer of the patient was analyzed by immunohistochemistry, which revealed loss of MSH2 expression in the tumors. To investigate whether the loss of MSH2 was a common feature of liposarcoma, we examined the MSH2 expression in an additional two sporadic liposarcomas, both of which were stained with anti-MSH2 antibody.

CONCLUSION: We identified a novel pathogenic germline mutation of *MSH2* in an HNPCC patient. Since an immunohistochemical analysis showed no nuclear staining for MSH2 protein in the liposarcoma as well as the rectal cancer, the loss of wild-type MSH2 protein was thus considered to possibly play a role in the development of liposarcoma in HNPCC patients.

(Am J Gastroenterol 2006;101:193–196)

INTRODUCTION

Hereditary nonpolyposis colorectal cancer (HNPCC) is an autosomal dominant disease characterized by the development of early onset neoplastic lesions including colorectal cancers without multiple polyps, as seen in familial adenomatous polyposis of the colon (1), and other HNPCC-associated tumors. HNPCC results from a germline mutation in such DNA mismatch repair genes such as *MSH2*, *MLH1*, and *MSH6* (2). The criteria for the clinical diagnosis for HNPCC were established as the Amsterdam criteria and were later revised as the Amsterdam criteria II (3). HNPCC-associated tumors were defined according to the Amsterdam criteria II as cancers that developed in the colon, endometrium, small bowel, ureter, and renal pelvis (4, 5), and included tumors in the stomach, ovarian, pancreas, biliary tract, and brain, and also sebaceous gland adenomas and keratoacanthomas based on the Bethesda guidelines (6). Soft tissue sarcoma is not a common feature of HNPCC, and it thus has been considered to be a coincidental tumor when it occurs in an HNPCC patient.

In this report, we describe an HNPCC patient who developed rectal cancer, cancer in the urinary bladder, and liposarcoma in his thigh. A genetic analysis identified a novel germline mutation in *hMSH2*. To investigate whether or not the inactivation of *MSH2* is involved in his liposarcoma, we analyzed the expression of MSH2 protein in the tumor by immunohistochemical staining. As a result, we detected no MSH2 expression in the tumor, thus suggesting that the absence of MSH2 might play a crucial role in the genesis of liposarcoma. Because little is known about the expression of MSH2 in sporadic liposarcomas, we further analyzed

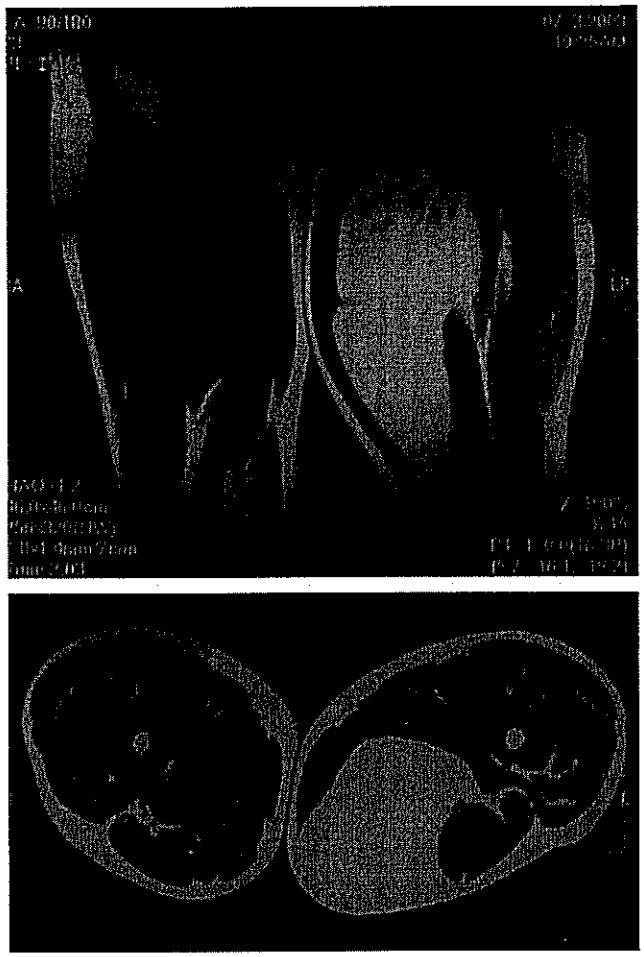

Figure 1. An MRI image of the soft tissue tumor in his left thigh.

two additional liposarcomas by immunohistochemical staining.

CASE REPORT

A 31-yr-old Japanese male patient underwent a transurethral operation for transitional cell cancer of urinary bladder. One year later, he underwent an anterior resection for rectal cancer. A pathological examination of the rectal cancer showed well-differentiated adenocarcinoma invading the muscularis propria without any lymph node metastasis. The tumor contained, in part, mucinous/signet-ring differentiation, and it was accompanied by infiltrating lymphocytes. A rapidly growing soft tissue tumor developed in his left thigh, when he was 40 yr old. Since MRI imaging suggested liposarcoma (Fig. 1), he underwent surgery to remove the tumor. The size of the tumor was 25 × 15 cm^2, and a histological examination diagnosed the tumor to be well-differentiated liposarcoma.

The early onset of his colorectal cancer and metachronous urinary tract cancer, and his family history thus suggested that he might have HNPCC syndrome (Fig. 2); his father had developed gastric cancer at 43 yr of age, and his paternal grandmother developed rectal cancer when she was in her sixties. Although these data did not fulfill the Amsterdam criteria II, they matched the revised Bethesda guidelines for the genetic testing for HNPCC. After obtaining the patient's written informed consent, a peripheral blood sample was taken to perform genetic testing. We analyzed the entire coding region of three mismatch repair genes including *MSH2*, *MLH1*, and *MSH6* by direct sequencing of the genomic DNA. As a result, we identified an AT deletion at codon 677 in exon 13 of *MSH2* (Fig. 3).

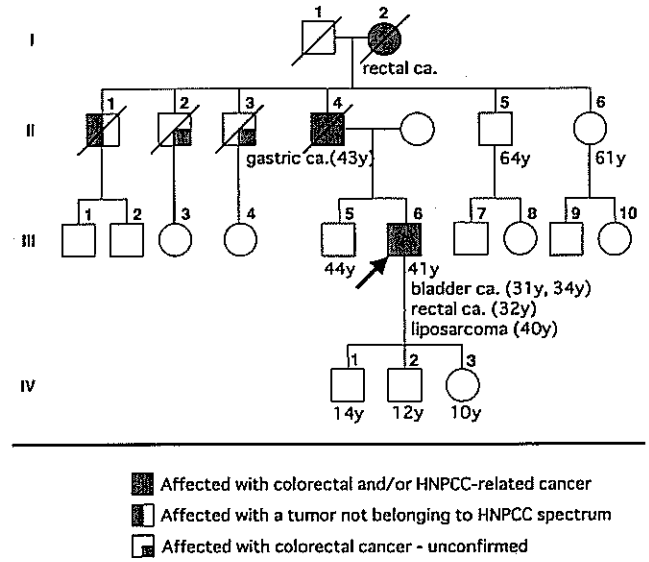

Figure 2. The family pedigree of the patient. The proband is indicated by an arrow. Age at diagnosis is shown in parenthesis.

To investigate whether *MSH2* is inactivated in the liposarcoma, paraffin-embedded tissue sections of the liposarcoma were examined by immunohistochemical staining using

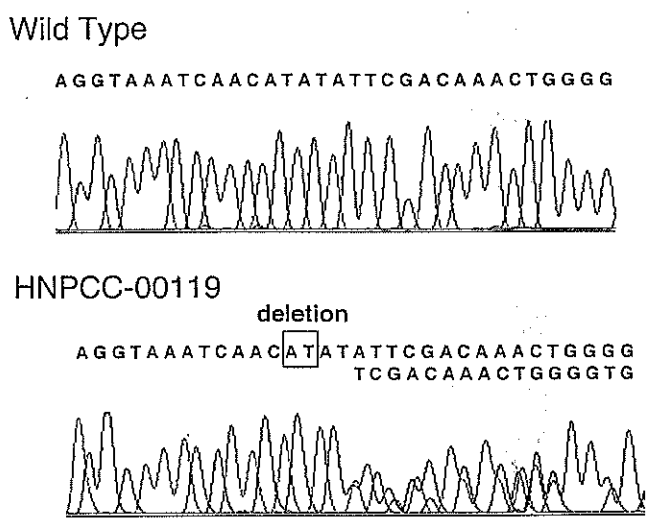

Figure 3. A two-base deletion (AT) in exon 13 in *MSH2*. The sequence of wild-type exon 13 of *hMSH2* in a healthy control (upper panel), and that of the mutant in the patient (lower panel).

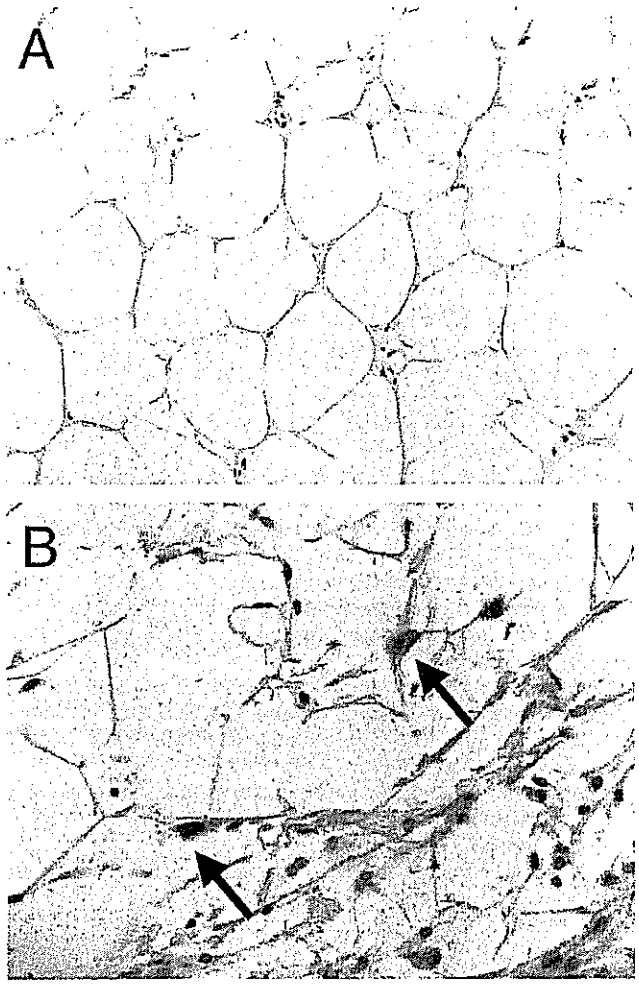

Figure 4. (*A*) Immunohistochemical staining of MSH2 in the liposarcoma specimen of the HNPCC patient. A sarcoma tissue specimen was MSH2 negative (original magnification × 200). (*B*) The expression of MSH2 in a sporadic liposarcoma. Nuclear staining is seen in the sarcoma cells (arrows, original magnification × 400).

anti-MSH2 antibody (clone G219-1129; PharMingen, BD Biosciences, San Diego, CA, USA). We used diaminobenzidine as the chromogen, and hematoxylin for counterstaining. As shown in Figure 4A, no MSH2 staining was observed in the nuclei of the sarcoma cells, thus indicating that both alleles of *hMSH2* was inactivated in the tumor cells. An immunohistochemical analysis of his metachronous rectal cancer tissue also revealed no nuclear staining for MSH2, while the normal mucosa of the adjacent rectum was positive for nuclear staining. These data suggested the loss of MSH2 expression to be involved in the liposarcoma as well as in the rectal cancer in the patient. We additionally studied the expression of MSH2 in two sporadic liposarcomas for comparison, because little is known about the expression of MSH2 protein in this type of tumor. In both liposarcomas, the tumor cells were positive for MSH2 (Fig. 4B).

DISCUSSION

In this study, we identified a novel germline mutation in MSH2, which has not yet been reported in either a previous study or exists in public databases, including the Human Mutation Database (http://www.hgmp.mrc.ac.uk/GenomeWeb/human-gen-db-mutation.html), and the International Society of Gastrointestinal Hereditary Tumors (InSIGHT: at http://www.insight-group.org/) database. The patient reported here did not fulfill the Amsterdam criteria II, but the clinicopathological data, patient's history, and family history satisfy the revised Bethesda Guidelines for identifying individuals at risk for HNPCC (6); The patient developed rectal cancer before 50 yr of age; His father had developed gastric cancer, and his paternal grandmother had developed rectal cancer; The synchronous rectal cancer specimen had a characteristic histology, such as mucinous/signet-ring differentiation and tumor-infiltrating lymphocytes. The information of his germline is thus expected to be helpful for future genetic testing of the family members. Since the two-base deletion at codon 677 predicted a premature stop codon and the consequent production of truncated MSH2 protein (a putative 697 amino acid-protein), the mutant protein should lose the wild-type MSH2 activity. As a result, we considered the deletion to be a pathogenic mutation. Notably, a nonsense mutation at codon 680 of MSH2 was reported in HNPCC patients (7, 8). A family member of a HNPCC patient with such a nonsense mutation developed gastric cancer (8). Since the truncation occurs at a similar position in the protein, the two forms of mutant protein may thus share similar properties.

HNPCC-associated tumors include colorectal, endometrial, stomach, ovarian, pancreas, ureter, renal pelvis, biliary tract, brain tumors, sebaceous gland adenomas and keratoacanthomas, and carcinoma of the small intestine. Sarcoma is not included in HNPCC-associated tumors. However, two reports revealed the occurrence of soft tissue sarcoma in HNPCC patients with a *hMSH2* mutation (9, 10), in which they suggested that sarcoma might form a part of the HNPCC spectrum; one was rhabdomyosarcoma (10) and the other was malignant fibrous histiocytoma (9). The presence of a germline mutation in *MSH2* is strongly associated with negative nuclear staining for MSH2 protein in HNPCC-associated intestinal cancer (11). We consistently found no nuclear MSH2 staining in the rectal cancer of our patient, thus implying that the wild-type allele had been lost in the cells. Since an immunohistochemical analysis also showed no MSH2 staining in the liposarcoma, wild-type MSH2 protein most likely became lost in the sarcoma cells too. Our data suggest that mismatch repair deficiency may play a role in the development of liposarcoma in patients with a *MSH2* mutation. An earlier article reported that no MSI was detected in 28 sporadic liposarcomas (12). However, Suwa and his colleagues found MSI in one out of seven liposarcomas in Japanese patients (13). Kawaguchi *et al.* recently reported that they observed MSI in 1 out of 12 liposarcomas, and that neither MLH1 nor MSH2 expression was observed in

the MSI-positive case (14). Therefore, the involvement of a deregulated mismatch repair mechanism in soft tissue sarcomas remains to be fully explored. Although two sporadic liposarcomas showed positive staining for MSH2 in our study, one should consider inactivation of other mismatch repair genes, such as *MLH1*, *MSH6*, and *PMS2*. The molecular etiology of HNPCC-associated tumors may differ from that of nonhereditary cases, as has been demonstrated for colorectal cancer (11, 15–17). Similarly, the genes involved in the development of liposarcoma in HNPCC patients may be different from those in sporadic liposarcoma. Alternatively, both HNPCC-associated and sporadic sarcoma may result from a common unknown genetic abnormality that is partially affected by the mismatch repair mechanism.

In summary, we found a novel pathogenic *MSH2* mutation in a patient with an early onset of rectal cancer, bladder cancer, and liposarcoma, and a family history of HNPCC-related tumors. An immunohistochemical analysis revealed an absence of wild-type MSH2 protein in both the liposarcoma as well as the rectal cancer. Our findings therefore appear to suggest the involvement of a mismatch repair deficiency in the development of liposarcoma in HNPCC patients.

ACKNOWLEDGMENT

This study was supported by a Grant-in-Aid for Cancer Research (13, 14) from the Minister of Health, Labour and Welfare (Chairman H. Ishikawa), Japan.

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Received June 9, 2005; accepted July 22, 2005.

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J Cancer Res Clin Oncol (2006) 132: 193–199
DOI 10.1007/s00432-005-0039-7

ORIGINAL PAPER

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Elevated risk of colorectal cancer associated with the AA genotype of the *cyclin D1* A870G polymorphism in an Indian population

Received: 20 June 2005 / Accepted: 30 August 2005 / Published online: 3 December 2005

Abstract *Purpose*: To investigate whether the common *cyclin D1* (*CCND1*) A870G polymorphism is a risk factor for colorectal cancer (CRC) in an Indian population. *Methods*: In this study, 301 newly diagnosed CRC patients and 291 healthy control subjects were genotyped by the PCR-RFLP method. Genotype frequencies were compared between cases and controls, and the association of genotypes with CRC was studied. *Results*: The *CCND1* 870 A allele was more frequently observed in CRC patients than controls (0.63 vs. 0.56, $P=0.01$), and after adjustment for age, sex, smoking habits, family history, family income and the consumption of meat, fish, vegetables and fruit, an increased risk was observed for the AA genotype compared to the GG+AG genotype (OR = 1.56; 95% CI: 1.10–2.21). The increased risk were also found for colon (OR = 1.96; 95% CI: 1.08–3.57) and rectal cancer (OR = 1.51; 95% CI: 1.04–2.19). No correlation was observed between genotypes and age of diagnosis of CRC (49.9, 48.7 and 49.4 years for the GG, AG and AA genotypes, respectively; $P=0.84$). Multivariate analysis also revealed a stronger positive association with the AA genotype among patients with high meat intake (OR = 2.67; 95% CI: 1.29–5.51), and particularly significant inverse associations with the GG+AG genotypes were also found for those with high vegetable consumption (OR = 0.46; 95% CI: 0.27–0.79 of 2–3 servings/day, and OR = 0.31; 95% CI: 0.18–0.53 for > 3 servings/day) and fish intake (OR = 0.48; 95% CI: 0.28–0.82). *Conclusion*: These data support the hypothesis that the *CCND1* A870G polymorphism may increase the risk of CRC in our Indian population.

Keywords Colorectal cancer · Cyclin D1 · A870G polymorphism

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Introduction

Cyclin D1, a protein encoded by the *CCND1* gene located on chromosome 11q13, is a key regulatory protein for the cell cycle transition from G1 phase to S phase (Sherr 1996), whose overexpression disrupts normal cell cycle control, possibly promoting the development and progression of cancers (Zhou et al. 1996; Wang et al. 1994; Donnellan and Chetty 1998). Furthermore, amplification of the *CCND1* gene and/or aberrant induction of cyclin D1 activity in colorectal cancer tissue have been found to be associated with enhanced cell proliferation and malignant progression in CRCs (Arber et al. 1996; McKay et al. 2000; Sutter et al. 1997; Bahnassy et al. 2004). A single nucleotide adenine-to-guanine substitution (A870G) in the splice donor region of exon 4 has been shown to influence the splicing variation coding for two mRNA transcripts. The G allele tends to produce mostly transcript-a, whereas the A allele is associated with the production of an aberrant splicing product termed

transcript-b which lacks an exon five sequence containing the PEST-rich region, which destabilizes the protein (Betticher et al. 1995). Therefore, A allele leads to a prolonged half-life and increases levels of cyclin D1 protein in cells, in turn promoting cell proliferation (Sawa et al. 1998).

A number of studies have linked the *CCND1* 870 A allele to increased cancer risk (Zhang et al. 2003; Buch et al. 2005; Shu et al. 2005; Shi et al. 2003; Wang et al. 2003; Wang et al. 2002), but the evidence is not entirely consistent (Yu et al. 2003; Ceschi et al. 2005; Cortessis et al. 2003; Forsti et al. 2004), and some controversy exists regarding the effects on CRC development (McKay et al. 2000; Kong et al. 2001; Porter et al. 2002; Le Marchand et al. 2003; Grieu et al. 2003). We have therefore evaluated links between the *CCND1* A870G polymorphism and susceptibility to CRC in an Indian population. In addition, we also investigated whether the association differs due to the location of tumors in either the colon or rectum, and whether the association is modified by dietary or environmental factors.

Materials and methods

Subject selection and data collection

The participants and data collection methods for this study have been described previously in detail (Jiang et al. 2005). Briefly, from 1999 to 2001, we recruited 301 colorectal cancer patients and 291 controls from Chennai and the surrounding area in southeastern India. Cases were recruited at the Madras Cancer Institute in Chennai, India, all enrolled patients with a first diagnosis of histologically confirmed colorectal cancer. Control subjects were cancer-free individuals, consisting of randomly selected attendants to patients having cancers other than CRC during the same time period of case collection. They were frequency-matched to case patients by sex and age (within 5 years). Informed consent was obtained from all study subjects. Trained interviewers collected information on socioeconomic status, medical history, alcohol, smoking, and tobacco chewing habits using a standard questionnaire. A 114 food –and- beverage item food-frequency questionnaire (FFQ) specific to this population was used to measure long-term intake of foods/food groups. All subjects were asked for their average frequency of consumption of food items per week over the past 1-year period (for cases, 1 year before the diagnosis of CRC). After the interview, a 7- ml blood sample was collected from each fasting subject. Soon after the blood sampling, blood was separated by centrifugation at 2,500 rpm for 15 min at 4°C and aliquoted into plasma (four tubes), buffy coat (one tube) and red blood cells (one tube), and immediately stored at −80°C. The study was approved by the internal review board of the Madras Cancer Institute in Chennai.

Genotyping

The DNA samples of the subjects were extracted from peripheral blood leukocytes using a GenTLE solution Kit (TaKaRa, Japan), and analyses were essentially carried out as previously described. (Betticher et al. 1995). To assess *CCND1* genotypes, a 167- bp fragment including the A870G polymorphism was amplified using forward and reverse primers (5′-GTG AAG TTC ATT TCC AAT CCG C-3′ and 5′-GGG ACA TCA CCC TCA CTT AC-3′, respectively). The A870G change creates a restriction site for the ScrF1 enzyme (New England Biolabs, Beverly, MA, USA) with the expected products after digestion with ScrF1 being 167 bp for AA, 145, 22 bp for GG, and 167, 145, 22 bp for AG. For quality-control purposes, 30 randomly selected DNA samples (5% of all samples) were determined by sequencing analysis using a BigDye Terminator Cycle Sequencing Kit, v 3.1 (Applied Biosystems, Foster City, CA, USA) with an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) (Kong et al. 2000). There were no discrepancies between the two results.

Statistical analysis

Differences in characteristics between cases and controls were assessed with the chi-square test, as well as disparities in genotype and allele frequencies. The Hardy–Weinberg equilibrium was checked with the chi-square test. One-way analysis of variance was employed to assess differences in age at diagnosis between genotypes in case subjects.

Unconditional logistic regression analyses were performed under a codominant model (risk differing across all three genotypes), a dominant model (subjects with one or two A alleles having the same increased risk) or a recessive model (only subjects with two A alleles at increased risk) to calculate odds ratios (ORs) and confidence intervals (95% CIs) for associations between genotypes and risk of CRC. To estimate dominant or recessive effects of the *CCND1* A870G genotype on CRC risk, log-likelihood statistics of nested and codominant models were compared. Adjustments were made for matching variables (age and sex) and for possible confounders. Covariates were identified as potential confounders by examining their distribution by case–control status. The BMI was excluded from covariates to avoid information bias, as it was affected by cancer in cases. The covariates were included in the model if they changed the ORs by more than 20% or significantly changed the likelihood ratio statistic ($P < 0.05$) on univariate analysis.

To examine the combined effects of *CCND1* A870G genotypes and certain risk factors, stratified analyses were conducted. Criteria for assessing effect modifiers were based on biological plausibility, and whether the risk estimation differed substantially across strata. The

likelihood ratio test was used to examine the interaction among variables with respect to the risk of CRC.

Calculations based on the prevalence of the A870G polymorphism and the size of our study population suggested 80% of power to detect an association at the 5% significance level (two-sided test) if the A870G polymorphism conferred at least a 1.8-fold increased risk (AA versus GG+AG genotypes). All statistical tests were two-sided and differences were considered to be statistically significant at $P < 0.05$. The SAS software (Version 8.20, SAS Institute Inc., Cary, NC, USA) was used for statistical analysis.

Results

Demographic and lifestyle characteristics for the 301 CRC cases and 291 control subjects are shown in Table 1. In general, the CRC cases had a smaller BMI, a lower family income, and a higher prevalence of family history of CRC, and also smoked more tobacco than the controls. In our population, after adjustment for sex, age, smoking habit, family history, family income, and the consumption of vegetables and fruit, a significant reduction of CRC risk ($P_{trend} = 0.001$ for vegetable intake, and $P_{trend} = 0.01$ for fruit intake) was found. Fish intake was related to a decreased risk of 0.63 (95% CI: 0.42–0.95), when comparing subjects who consumed ≥2 servings/week with those consuming <2 servings/week. In contrast, high meat intake (≥2 servings/week) relative to low meat intake (<2 servings/week) conferred an increased risk (OR = 1.45, 95% CI: 0.92–2.35).

Data for genotype frequencies and associations between *CCND1* A870G polymorphism and risk of CRC are shown in Table 2. The distribution of the observed genotypes did not deviate from the Hardy–Weinberg equilibrium for the A870G polymorphism ($P = 0.21$ in cases, and $P = 0.86$ in controls). The GG, AG, and AA genotype frequencies were 15.3, 43.2, and 41.5%, respectively in the cases, compared with 19.3, 49.8, and 30.9% for the controls, with a statistically significant difference between the two ($P = 0.03$). The A allele frequency for the A870G polymorphism was greater among cancer patients than controls (0.63 vs. 0.56, $P = 0.01$). There were no significant differences in the genotype frequencies between male and female case subjects. Age at diagnosis did not appreciably vary among the three genotypes (AA: 49.4 years, AG: 48.7 years, GG: 49.9 years, $P = 0.84$).

After adjustment for sex, age, smoking habit, family history, family income, and the consumption of meat, fish, vegetables and fruit, the OR was 1.04 (95% CI: 0.66–1.67) for the AG genotype, and 1.64 (95% CI: 1.02–2.66) for the AA genotype compared to the GG genotype. With the GG genotype as reference, the OR for the combined AG and AA genotypes together was 1.24 (95% CI: 0.80–1.93). Comparing the dominant model with the codominant model, the χ^2 value of was 5.73 ($P = 0.02$), so the dominant model was not applicable. Using the combined GG and AG genotypes as the reference, we found the OR to be 1.56 (95% CI: 1.10–2.21) for the AA genotype, and the χ^2 value of the recessive model was 0.14 ($P = 0.71$) against the codominant model. These results suggested that the effects of the A allele better fit a recessive model than a dominant model. Although the above associations were essentially the same for rectal cancers analyzed separately, a slightly stronger effect for the AA genotype was found for colon cancer (OR = 1.96; 95% CI: 1.08–3.57) in the recessive model.

Table 3 presents data for potential interactions between the *CCND1* A870G genotype and dietary or environmental factors with regard to the development of CRC. A significantly elevated risk was found for patients with the AA genotype who consumed more meat (OR = 2.67; 95% CI: 1.29–5.51). In addition, significant inversed associations were found for high vegetable (OR = 0.46; 95% CI: 0.27–0.79 for 2–3 servings/day, and OR = 0.31; 95% CI: 0.18–0.53 for >3 servings/day) or fish intake (OR = 0.48; 95% CI: 0.28–0.82) among those with GG+AG genotypes. However, there were no statistically significant interactions between these risk factors and the A870G polymorphism with regard to overall CRC risk.

Discussion

The present investigation, conducted to explore the association between the *CCND1* A870G polymorphism and colorectal cancer in an Indian population, demonstrated a significant 1.56-fold increase in the OR for the AA genotype related to the influence of the polymorphism fitting a recessive model of inheritance. Furthermore, the association with the AA genotype was found to be slightly stronger for colon than rectal cancer.

Cyclin D1 is a protein derived from the *CCND1* gene, which is involved in the cell cycle in neoplasia as well as normal tissue. In the G1 phase of the cell cycle, cyclin D1 together with its cyclin dependent kinase partner is responsible for transition to the S phase (Sherr 1996). Cyclin D1 protein is abundant in 30–70% of colorectal cancers (Arber et al. 1996; McKay et al. 2000; Bahnassy et al. 2004); additionally, about 50% of the colon carcinomas display a two to fivefold increase in the expression of Cyclin D1 mRNA and protein when compared with the paired normal mucosa samples (Sutter et al. 1997). Moreover, antisense *CCND1* complementary DNA suppresses the proliferation of human colon cancer cells as well as CRC tumorigenesis in nude mice (Arber et al. 1997). The A870G polymorphism modulates the alternative splicing pattern of the CCND1 mRNA, with the transcript-b preferentially transcribed from the A allele resulting in an increase in cyclin D1 protein levels (Betticher et al. 1995; Sawa et al. 1998). In patients with squamous cell carcinoma of the head and neck, the *CCND1* GG genotype was associated with a significantly reduced cyclin D1 protein expression

Table 1 Characteristics of the colorectal cancer patients and control subjects

	Cases (%) ($n=301$)	Controls (%) ($n=291$)	P
Males	196 (65.1)	183 (62.9)	NS
Age (year)			
20–44	107 (35.6)	111 (38.1)	NS
45–59	109 (36.2)	121 (41.6)	
60–75	85 (28.2)	59 (20.3)	
BMI (kg/m^2)			
< 20.0	153 (50.8)	109 (37.5)	< 0.01
20–24.9	110 (36.6)	111 (38.1)	
≥25	38 (12.6)	71 (24.4)	
Education (year)			
< 5	104 (34.5)	88 (30.2)	NS
5–11	155 (51.5)	163 (56.0)	
> 11	42 (14.0)	40 (13.8)	
Religion			
Hindu	265 (88.0)	256 (88.0)	NS
Muslim	23 (7.7)	27 (9.3)	
Christian	13 (4.3)	8 (2.7)	
Family income (rupees/week)			
≤ 500	143 (47.5)	97 (33.3)	< 0.05
501–1300	69 (22.9)	101 (34.7)	
> 1300	89 (29.6)	93 (32.0)	
Smoking habit (pack–years)			
0	240 (79.7)	227 (78.0)	< 0.01
≤ 10	41 (13.6)	58 (19.9)	
> 10	20 (6.7)	6 (2.1)	
Drinking habit (years)			
0	245 (81.4)	235 (80.8)	NS
< 20	38 (12.6)	33 (11.3)	
≥20	18 (6.0)	23 (7.9)	
Tobacco chewing habit	39 (13.0)	28 (9.6)	NS
Family history of CRC	4 (1.3)	0	< 0.05
Vegetable intake (servings/day)			
< 2	117 (38.9)	65 (22.3)	< 0.01
2–3	109 (36.2)	111 (38.2)	
> 3	75 (24.9)	115 (39.5)	
Fruit intake (servings/week)			
≤ 4	132 (43.8)	102 (35.1)	< 0.05
≤ 8	126 (41.9)	129 (44.3)	
> 8	43 (14.3)	60 (20.6)	
Meat intake (servings/week)			
< 2	236 (78.4)	237 (81.4)	NS
≥2	65 (21.6)	54 (18.6)	
Fish intake (servings/week)			
< 2	251(83.4)	219 (75.3)	< 0.05
≥2	50 (16.6)	72 (24.7)	

* Examined by the chi-square test

(Holley et al. 2001). However, the *CCND1* A870G polymorphism was unrelated to the expression in patients with CRC (McKay et al. 2000). Since little is known about the association between *CCND1* A870G polymorphism and cyclin D1 protein expression to date, further studies should be conducted.

Several studies have been conducted on the influences of the *CCND1* A870G polymorphism on the development of CRCs or sporadic colorectal adenomas, and three showed statistically significantly positive links between the A allele or the AA genotype and susceptibility. The first, a hospital- based study of 156 sporadic CRC patients and 152 controls, revealed an OR of 2.68 (95% CI: 1.38–5.19) for the AA genotype, while the AG genotype was unrelated to risk (OR = 1.06, 95% CI: 0.62–1.81), suggesting a recessive model (Kong et al. 2001). In the second, Porter et al. found the dominant A allele to be associated with an increased risk of familial and sporadic CRC, but in the latter case statistical significance was not attained ($P=0.08$) (Porter et al. 2002). The third study by Marchand et al. recently demonstrated a 50% enhanced risk of CRC for the AA genotype, with a statistically significant gene-dosage effect ($P=0.03$) in a large population-based study, and the relation with the A allele was significantly stronger for an advanced disease stage and rectal cancer (Le Marchand et al. 2003). Two further studies, one on HNPCC family members carrying mutations (49 cases and 37 controls), and the other on Finnish HNPCC family mutation carriers (146 cases and 186 controls), provided evidence that the A allele and the presence of a variant transcript-b are related to a significantly lower age for

Table 2 Odds ratios and 95% CIs for colorectal cancer with reference to the *CCND1* A870G genotype

	No.	All cases		No.	Colon cancer		No.	Rectal cancer		Controls No.
		OR (95% CI)			OR (95% CI)			OR* (95% CI)		
		Crude	Adjusted		Crude	Adjusted		Crude	Adjusted	
Codominant inheritance										
GG	46	1.00 (reference)	1.00 (reference)	8	1.00 (reference)	1.00 (reference)	38	1.00 (reference)	1.00 (reference)	56
AG	130	1.09 (0.69–1.72)	1.04 (0.66–1.67)	23	1.11 (0.47–2.63)	1.16 (0.46–2.91)	107	1.09 (0.67–1.76)	1.00 (0.61–1.65)	145
AA	125	1.69 (1.05–2.72)	1.64 (1.02–2.66)	28	2.18 (0.93–5.11)	2.19 (0.87–5.48)	97	1.59 (0.96–2.62)	1.51 (0.90–2.58)	90
Dominant inheritance										
GG	46	1.00 (reference)	1.00 (reference)	8	1.00 (reference)	1.00 (reference)	38	1.00 (reference)	1.00 (reference)	56
AA+AG	255	1.32 (0.86–2.03)	1.24 (0.80–1.93)	51	1.52 (0.68–3.38)	1.55 (0.66–3.67)	204	1.28 (0.81–2.01)	1.19 (0.75–1.90)	235
Recessive inheritance										
GG+AG	176	1.00 (reference)	1.00 (reference)	31	1.00 (reference)	1.00 (reference)	145	1.00 (reference)	1.00 (reference)	201
AA	125	1.59 (1.13–2.22)	1.56 (1.10–2.21)	28	2.02 (1.14–3.56)	1.96 (1.08–3.57)	97	1.49 (1.05–2.14)	1.51 (1.04–2.19)	90

* Adjusted for sex, age, smoking habit, family history, family income, and consumption of meat, fish, vegetables, and fruit

the onset of CRC (Kong et al. 2000; Bala and Peltomaki 2001). Although other studies generated negative results for links between the A870G polymorphism and CRC risk (McKay et al. 2000; Grieu et al. 2003), an increased level of cyclin D1 protein was positively associated with reduced survival time (McKay et al. 2000). While Lewis et al reported an overall 50% increase in risk for sporadic colorectal adenomas apparent for individuals with the A allele (Lewis et al. 2003), Crabtree et al. could not establish any effect on severity of colonic familial adenomatous polyposis (Crabtree et al. 2004). Overall, however, the findings suggest that *CCND1* A870G polymorphism indeed has an influence on the susceptibility to FAP, HNPCC, sporadic CRC and sporadic colorectal adenomas.

Our findings on CRC in this Indian population were generally in agreement, but in contrast to the dominant or gene-dosage effects on CRC in two studies (Porter et al. 2002; Le Marchand et al. 2003). We found the influence of the A allele fitted a recessive model of inheritance, consistent with Kong's results and investigations on other types of tumors (Kong et al. 2001; Shi et al. 2003; Wang et al. 2002, 2003). Variations in the possible interactions of the A allele with other genetic or environmental factors may explain, to some extent, the observed differences. The A allele may be associated with

Table 3 Associations of colorectal cancer with selected risk factors by the *CCND1* A870G genotype

	Genotypes				P for interaction
	GG+AG		AA		
	Cases/Controls	OR* (95% CI)	Cases/controls	OR* (95% CI)	
Current smokers					
No	145/156	1.00 (reference)	95/71	1.39 (0.94–2.06)	0.20
Yes	31/45	0.68 (0.38–1.22)	30/19	1.64 (0.85–3.20)	
Current drinkers					
No	140/156	1.00 (reference)	105/79	1.43 (0.98–2.10)	0.43
Yes	36/45	0.89 (0.52–1.53)	20/11	2.11 (0.95–4.70)	
Family history of CRC					
No	173/201	1.00 (reference)	124/90	1.56 (1.10–2.21)	0.99
Yes	3/0	NA	1/0	NA	
Vegetable intake (servings/day)					
<2	69/41	1.00 (reference)	48/24	1.14 (0.60–2.15)	0.39
2–3	61/77	0.46 (0.27–0.79)	48/34	0.80 (0.44–1.46)	
>3	46/83	0.31 (0.18–0.53)	29/32	0.53 (0.27–1.01)	
Fruit intake (servings/week)					
≤4	78/68	1.00 (reference)	54/34	1.34 (0.77–2.34)	0.91
≤8	72/93	0.67 (0.42–1.07)	54/36	1.31 (0.75–2.28)	
>8	26/40	0.58 (0.31–1.10)	17/20	0.71 (0.33–1.51)	
Meat intake (servings/week)					
<2	141/159	1.00 (reference)	95/78	1.33 (0.90–1.97)	0.09
≥2	35/42	0.89 (0.52–1.52)	30/12	2.67 (1.29–5.51)	
Fish intake (servings/week)					
<2	148/145	1.00 (reference)	103/74	1.35 (0.91–1.99)	0.16
≥2	28/56	0.48 (0.28–0.82)	22/16	1.24 (0.61–2.53)	

* Adjusted for sex, age, smoking habit, family history, family income, and consumption of meat, fish, vegetables and fruit
NA not available

a significantly lower age of onset of hereditary nonpolyposis colorectal cancer in mismatch repair gene mutation carriers (Kong et al. 2000), but age influence was not noted here or elsewhere (Kong et al. 2001; Porter et al. 2002; Le Marchand et al. 2003). Patients carrying such genetic abnormality may be more sensitive to the effects of the *CCND1* A870G polymorphism (Kong et al. 2000), the low frequency of the mismatch repair gene mutations in sporadic CRC patients possibly explained our null findings. An important finding in our study was the elevated risk for patients with the AA genotype who consumed greater amounts of meat; conversely those with the G allele, demonstrated lower risk if their intake of vegetable or fish was high. Although not statistically significant overall, there were patterns suggesting that dietary factors may modify the associations of the *CCND1* A870G polymorphism with CRC risk.

In this first study of associations between the *CCND1* A870G polymorphism and CRC in an Indian population, we found the frequency of the Ala allele to be similar to those reported for Chinese and Native Hawaiians (0.51–0.58) (Zhang et al. 2003; Shu et al. 2005; Yu et al. 2003; Ceschi et al. 2005; Le Marchand et al. 2003), but higher than for Japanese and Caucasians (0.43) (Wang et al. 2002, 2003; Kong et al. 2001; Le Marchand et al. 2003). Potential limitations of the present study should be considered. First, while it is known that the *CCND1* 870A allele significantly correlates with advanced tumor stage and survival (McKay et al. 2000; Le Marchand et al. 2003), we were not able to collect pathological and survival information. Second, since the exposure information was collected after the diagnosis of CRC, differential dietary recall between cases and controls could yield a certain information bias. Third, our sample size was relatively small for stratified analyses to explore the gene–environment or gene–diet interactions, resulting in insufficient statistical power for interaction tests, so that further future larger studies are clearly warranted.

In conclusion, our present investigation indicated that the CCND1 870 AA genotype is associated with an increased CRC risk, with the A allele acting as a recessive genetic susceptibility factor in this Indian population. Our findings also suggested that *CCND1* A870G polymorphism might differentially influence the impact of dietary risk factors.

Acknowledgements This work was supported in part by the International Scientific Research Program, Special Cancer Research of the Japanese Ministry of Education, Culture, Sports, Science and Technology. We thank Drs. V. Shanta and. T. Rajkumar at the Cancer Institute, Chennai, India for their cooperation.

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