RESEARCH ARTICLE

Risk Factors for Rectal Cancer and Methylenetetrahydrofolate Reductase Polymorphisms in a Population in Northeast Thailand

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Abstract

Background and Aim: Polymorphisms in methylenetetrahydrofolate reductase (*MTHFR*) are known to be associated with predisposition for certain cancers. This study aimed to evaluate the effects of lifestyle factors, family history and genetic polymorphisms in *MTHFR* C677T and A1298C on rectal cancer risk and possible interactions with lifestyle factors in Northeast Thailand. Methods: A hospital-based case-control study was conducted during 2002-2006 with recruitment of 112 rectal cancer cases and 242 non-rectal cancer patient controls. Information was collected using a structured-questionnaire. Blood samples were obtained for assay of *MTHFR* C677T and A1298C genotypes by polymerase chain reaction with restriction fragment length polymorphism (PCR-RFLP) techniques. Associations between lifestyle factors, family history and genetic polymorphisms v.s. rectal cancer risk were assessed using logistic regression analysis. Results: Subjects with frequent and occasional constipation had a higher risk ($OR_{adj.}$=14.64; 95%CI=4.28-50.04 and $OR_{adj.}$=2.15; 95%CI=1.14-4.06), along with those who reported ever having hemorrhoids ($OR_{adj.}$=2.82; 95%CI=1.36-5.84) or a family history of cancer ($OR_{adj.}$=1.90; 95%CI=1.06-3.39). Consumption of a high level of pork was also associated with risk ($OR_{adj.}$=1.82; 95%CI=1.05-3.15). Interactions were not observed between *MTHFR* and other risk factors. Conclusions: This study suggested that the risk factors for rectal cancer in the Thai population are bowel habits, having had hemorrhoids, a family history of cancer and pork consumption.

Keywords: Risk factors - methylenetetrahydrofolate reductase - polymorphisms, rectal cancer

Asian Pacific J Cancer Prev, **13**, 4017-4023

Introduction

Rectal cancer is rare in developing countries, in contrast to the high incidence rates in Europe, North America, Australia and Japan. Significant differences also exist within continents (Curado et al., 2007). In Thailand, the estimated total of 4,789 new cases of rectal cancer in 1999 had a male : female ratio of 1.25:1. The annual age-standardized incidence rates of rectal cancer in Thailand were 3.6 and 2.7 per 100,000 in males and females, respectively (Khuhaprema et al., 2007). The highest incidence rates for males were in Bangkok, Chiang Mai and Rayong with ASR of 4.7, 4.6 and 4.6, respectively. Meanwhile, Songkhla, Chiang Mai and Bangkok had the highest incidence rates in females with the ASR of 3.6, 3.6 and 3.3, respectively (Khuhaprema et al., 2007).

There are a number of behavioral or environmental factors that increase risk of colorectal cancer (Potter et al., 1993; Suwanrungruang et al., 2006; Sriamporn et al., 2007). Among the most consistent risk factors are a diets with a low fiber contents, and higher intake of calories and fat, and especially of red meat. In addition, obesity, sedentary lifestyles and alcohol consumption have been implicated as potential risk factors (Potter and McMichael, 1986; Giovannucci et al., 1995). In epidemiologic studies, low-folate diets have been found to increase the risk of colorectal cancer (Benito et al., 1990; Glynn et al., 1996; Sharp et al., 2008). Other dietary factors, including consumption of methionine, vitamin B6, B12, and alcohol have been associated with colorectal cancer in some studies but not all epidemiologic studies (Giovannucci et al., 1993; Giovannucci et al., 1995; Slattery et al., 1997; Sharp et al., 2008). However, the underlying mechanisms of these dietary factors in relation to rectal cancer are not clear.

Methylenetetrahydrofolate reductase (*MTHFR*) is an important enzyme in folate metabolism; it catalyzes the conversion of 5,10-methylenetetrahydrofolate

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(5,10-methylene-THF) to 5-methyltetrahydrofolate (5-methyl-THF) (Choi and Mason, 2002). Two common polymorphisms in *MTHFR* gene have been characterized (Frosst et al., 1995; Weisberg et al., 1998). These polymorphisms include the C677T polymorphism codes for an alanine to valine substitution in the N-terminal catalytic domain, and the A1298C polymorphism codes for an alanine to glutamine substitution in the C-terminal regulatory domain (Weisberg et al., 1998). A common mutation (C677T) causes reduced enzyme activity, leading to lower levels of circulating folate (5-methyl-THF), an accumulation of 5,10-methylene-THF, and increased plasma homocystein levels (Jacques et al., 1996; Ma et al., 1996).

Functional polymorphisms in *MTHFR* and their associations with cancer risk are of great interest. Indeed, numerous epidemiologic studies have examined the relationship between *MTHFR* polymorphisms and cancer risk but have generated conflicting results. Several studies have shown that the low-activity variant of *MTHFR* C677T was associated with a decreased risk for colorectal cancer (Ma et al., 1997; Chen et al., 1999), colon cancer (Slattery et al., 1999) and acute lymphocytic leukemia (Skibola et al., 1999), In contrast, the same variant has also been related to an increased risk for various cancers, including endometrial cancer (Esteller et al., 1997), cervical intraepithelial neoplasia (Piyathilake et al., 2000), esophageal squamous cell carcinoma (Song et al., 2001), gastric cancer (Shen et al., 2001), and bladder cancer (Lin et al., 2004).

Although various epidemiologic studies have been examining the association between *MTHFR* polymorphisms and colorectal cancer risk, few colon and rectal cancer studies have analyzed them separately, and results have been the conflicting. Importantly, no investigations have examined *MTHFR* 677 and 1298 genotypes on the effects of potential risk factors of rectal cancer among Thai people.

As part of a multi-centre study of "The epidemiologic study of host and environmental factors for stomach and colon cancers in Southeast Asian Countries" which was approved by the Research Ethics Committee, Faculty of Medicine, Khon Kaen University, Reference No. HE450818, we examine the risk factors for rectal cancer in lifestyle, environment and genetic factors in the population of Northeast Thailand.

Materials and Methods

A hospital-based case-control study was conducted to examine the risk factors for rectal cancer in both lifestyle and genetic factors. Cases of rectal cancer and controls were studied on the *MTHFR* polymorphisms and lifestyle factors, including dietary intake, smoking, alcohol drinking, occupation, defecation and family history of cancer.

Subjects

112 new cases, histologically diagnosed as rectal cancer (87 rectum, NOS (C20.9) and 25 rectosigmoid junction (C19.9)) were recruited from Srinagarind Hospital and Khon Kaen Regional Hospital, Khon Kaen Province, between October 2002 and October 2006. All were from Khon Kaen Province or neighbouring provinces. All cases were interviewed within 3 months of first diagnosis. In the same period, 242 patients from the same hospitals were recruited as controls, frequency-matched to cases of large bowel cancer, by sex and age group. The controls had a variety of diseases, the main ones being diseases of the eye, genito-urinary system or infection/inflammation. Subjects with gastrointestinal disease or other cancers were excluded. All subjects gave informed consent in writing to their participation in the study. Subjects (about 10% of cases and 10% controls) who refused or were too old or unable to do the interview were excluded from the study. The 5 ml. blood samples obtained from cases and controls were transferred to the laboratory for analysis of *MTHFR* polymorphisms.

Interview

Subjects were interviewed by two trained interviewers, using a structured questionnaire. The questionnaire was composed of two sections. The first section included demographic and socio-economic status, smoking history (allowing for various periods of different consumption) and family history of cancers. The second section was a food frequency questionnaire structured by meals. The interview referred to dietary habit before the subjects became sick with their present illness (one year earlier). All subjects were reminded of this condition throughout the interview.

Laboratory methods

Genomic DNA was extracted from buffy coat fraction of the rectal cancer cases and their controls using the standard technique of Nagoya City University Medical School, Nagoya, Japan. Gene amplification and polymorphism analyses were performed in the Microbiological Laboratory at the Faculty of Medicine, Khon Kaen University, Thailand.

The technique of polymerase chain reaction with restriction fragment length polymorphism (PCR-RFLP) was modified as previously described as an amplification of *MTHFR* C677T, and A1298C polymorphisms were modified as previously described (Frosst et al., 1995; Weisberg et al., 1998). More details of digestion products and evaluation methods can be found elsewhere (Promthet et al., 2010).

Statistical analysis

The association between rectal cancer and major risk factors was evaluated using odds ratios (ORs) and their 95% confidence intervals (95%CIs) derived from the logistic regression analysis. Crude and adjusted odds ratios were estimated for each independent variable. Logistic regression model was used to evaluate the multiplicative interaction effects. Factors found having a strong association with rectal cancer by univariate analysis, and factors without association by univariate analysis but reported having an important role as factors related to risk of rectal cancer in the literature, were included in the multivariate analysis. The possible effect modifications

by *MTHFR* C677T and A1298C on the effects of major risk factors for rectal cancer were analyzed by logistic regression model.

The distribution of alleles/genotypes of *MTHFR* polymorphisms in the rectal cancer cases and controls was examined using χ^2 test; 2x2 tables were used to compare genotype distribution between the 2 respective groups. Both tests were utilized to compare the cases and control subjects with regard to genotype frequencies and potential risk factors for rectal cancer such as demographic characteristics, diet, smoking and alcohol drinking.

ORs were adjusted for age and sex according to the polymorphisms in *MTHFR* C677T and A1298C genes with risk factors for rectal cancer. Statistical analyses were performed using STATA version 10. A probability level (P-value) of less than 0.05 was used as a criterion of significance.

For smoking, smokers included those who smoked filtered, unfiltered cigarettes and yamuan (a home-made cheroot). Ex-smokers were categorized as smokers. Duration of smoking, and average number of cigarettes per year were computed based on all smoking periods reported and dichotomized on the median value of the controls. The average number of cigarettes was calculated as annual cigarette consumption (filtered and unfiltered) plus 1.5 times the annual yamuan consumption. The 1.5 correction factor was used to allow for the longer size of yamuan compared with the regular cigarettes. The amount of cigarettes was categorized based on the 50th percentile of the controls and dichotomized into low and high levels.

For alcohol drinking, ever drinkers, were defined as those who consumed at least one type of any alcoholic beverage (beer, sato, white whisky, maekong or other whiskies) and consumed within a range of everyday to once a month. Those who did not drink or consume any alcoholic beverage with a frequency of less than once a month were categorized as nondrinkers. The average amount of alcohol consumption was analyzed based on gram per day, i.e., net alcohol consumption measure and percentile. Alcohol volume (%Vol.) of beer was defined as 5.0%, sato as 7.0%, white whisky as 40% and red whisky as 35%. Average amount of alcohol consumption was calculated and then converted as net alcohol per day into 3 categories including non-drinker, ≤0.50 and >0.50 net alcohol per day.

Dietary intakes within the previous year (beef, pork, poultry, freshwater and saltwater fish/shellfish, offal, vegetables and fruit), were categorized into two levels as low and high. Frequency and the amount of dietary intakes per year were computed and dichotomized as low and high consumption on the median value of the controls.

Results

There were 112 cases (58 males and 54 females) with a median age of 57 years and 242 controls (129 males and 113 females) with a median age of 55 years. Most of the subjects were farmers and educated from primary school.

Among the 354 subjects, cases (112) and controls (242) were genotyped for *MTHFR* C677T. The prevalence of T allele was 17.0% in cases and 23.6% in controls: 83.0% and 76.5% were C/C homozygotes, 16.1% and 20.3% were C/T heterozygotes and the remaining 0.9% and 3.3% were T/T homozygotes in cases and controls, respectively. The corresponding values for the 354 individuals with *MTHFR* A1298C data were as follows: 33.0% and 35.1% A/A homozygotes, 66.1% and 60.7% A/C heterozygotes and 0.9% and 4.1% C/C homozygotes, respectively, with a C allele prevalence of 67.0% and 64.8% in cases and controls, respectively. There was no significant interaction between the polymorphisms of *MTHFR* C677T and *MTHFR* A1298C on rectal cancer risk (P-values for interaction=0.548).

Table 1 shows the association of polymorphisms in *MTHFR* and rectal cancer by univariate analysis. Genotype frequencies of two genes were distributed according to Hardy-Weinberg equilibrium. The subjects with heterozygotes and homozygotes variant genotypes, both individual and combined were compared with homozygotes wild-type genotype in each variant. These results were not significantly different from all genotype subgroups of two genes.

In the univariate analysis (Table 2), subjects who reported having occasional constipation and frequent constipation had a higher risk of rectal cancer than those who had normal defecation (OR=2.80; 95%CI=1.69-4.64 and OR=22.14; 95%CI=7.22-67.86, respectively). Those who reported having a history of hemorrhoids had a statistically significantly higher risk of rectal cancer with statistical significance (OR=3.79; 95%CI=2.24-6.43). Those who reported having relatives with any type of cancer had a higher risk of rectal cancer than those with no cancer in the family (OR=1.83; 95%CI=1.13-2.97). Those who consume alcoholic beverage less than once a month seemed to have a protective factor against rectal cancer (OR=0.42; 95%CI=0.19-0.90). No differences were noted in the rectal cancer risk between tea or coffee drinkers and non-drinkers. There was no association between smoking habit and risk of rectal cancer. For types of dietary intake based on the food frequency questionnaire, using the low level as a referent group, there was an association between a high level of pork consumption and rectal cancer risk (OR=1.97; 95%CI=1.23-3.15). Beef consumption showed a higher risk for rectal cancer but was not of statistical significance.

In the multivariate analysis (Table 2) shows the adjusted OR and 95%CI. The bowel habit, those who had occasional or frequent constipation had a high risk of rectal cancer (OR=2.15, 95%CI=1.14-4.06 and OR=14.64, 95%CI=4.28-50.04, respectively). Hemorrhoids were

Table 1. Univariate Analysis of Polymorphisms in *MTHFR* and Rectal Cancer in Northeast Thailand

Variables	Cases		Controls		OR	95%CI	P-value
	n	%	n	%			
MTHFR C677T polymorphism							
C/C	93	83.0	185	76.5	1		
C/T, T/T	19	17.0	57	23.5	0.66	0.37-1.18	0.162
MTHFR A1298C polymorphism							
A/A	37	33.0	85	35.1	1		
A/C, C/C	75	67.0	157	64.9	1.1	0.68-1.76	0.701

Table 2. Univariate Analysis of Potential Risk Factors for Rectal Cancer in Northeast Thailand

Variables		Cases (n)	Controls (n)	OR[a]	OR[b]	95%CI	P-value
Bowel habits:	Normal	40	161	1	1		
	Occasional constipation	48	69	2.80	2.15	1.14-4.06	0.019
	Frequent constipation	22	4	22.14	14.64	4.28-50.06	<0.001
Hemorrhoids:	No	67	201	1	1		
	Yes	43	34	3.79	2.82	1.36-5.84	0.005
Family history of cancer:	No	71	184	1	1		
	Yes	41	58	1.83	1.9	1.06-3.39	0.031
Frequency of alcohol consumption							
	Nondrinker	65	132	1	1		
	<1/month	9	44	0.42	0.59	0.22-1.57	0.288
	Weekly	15	30	1.02	2.00	0.77-5.18	0.153
	Daily	23	36	1.30	1.89	0.78-4.62	0.16
Pork (average times/day):	Low (≤0.5)	65	177	1	1		
	High (>0.5)	47	65	1.97	1.82	1.05-3.15	0.032
Beef (average times/day):	Low (≤0.08; or ≤2.4 times/month)	75	181	1	1		
	High (>0.08; or >2.4 times/month)	36	61	1.42	1.75	0.97-3.15	0.063

[a]Crude odds ratio; [b]Adjusted for age, sex and variables in table

Table 3. Gene-Environmental Interactions of *MTHFR* C677T and A1298C Polymorphisms with Potential Risk Factors for Rectal Cancer in Northeast Thailand

MTHFR	Environment/lifestyle factors	Cases (n)	Controls (n)	Adjusted OR	95%CI[a]	P-value[b]
C677T	Bowel habits					0.586
C/C	Normal	32	127	1		
C/C	Occasional constipation	40	49	3.24	1.83-5.73	
C/C	Frequent constipation	19	3	25.14	7.00-90.21	
C/T, T/T	Normal	8	34	0.93	0.39-2.21	
C/T, T/T	Occasional constipation	8	20	1.59	0.64-3.93	
C/T, T/T	Frequent constipation	3	1	11.91	1.20-118.30	
C677T	Family history of cancer					0.422
C/C	No	61	141	1		
C/C	Yes	32	44	1.68	0.97-2.90	
C/T, T/T	No	10	43	0.54	0.25-1.14	
C/T, T/T	Yes	9	14	1.49	0.61-3.62	
C677T	Alcohol drinking					0.875
C/C	No	54	100	1		
C/C	Yes	39	85	0.85	0.51-1.41	
C/T, T/T	No	10	29	0.64	0.28-1.41	
C/T, T/T	Yes	9	28	0.59	0.26-1.35	
A1298C	Bowel habits					0.159
A/A	Normal	8	58	1		
A/A	Occasional constipation	19	24	5.74	2.21-14.89	
A/A	Frequent constipation	8	1	58.0	6.39-526.79	
A/C, C/C	Normal	32	103	2.25	0.97-5.21	
A/C, C/C	Occasional constipation	29	45	4.67	1.95-11.20	
A/C, C/C	Frequent constipation	14	3	33.83	7.94-144.18	
A1298C	Family history of cancer					0.409
A/A	No	25	63	1		
A/A	Yes	12	22	1.37	0.59-3.19	
A/C, C/C	No	46	121	0.96	0.54-1.70	
A/C, C/C	Yes	29	36	2.03	1.03-3.98	
A1298C	Alcohol drinking					0.706
A/A	No	19	43	1		
A/A	Yes	18	42	0.97	0.45-2.10	
A/C, C/C	No	45	86	1.18	0.62-2.27	
A/C, C/C	Yes	30	71	0.96	0.48-1.90	

[a]95%CI for adjusted OR; [b]P-value for interaction

a significant rectal cancer risk factor (OR=2.82, 95%CI=1.36-5.84). Subjects who had family history of cancer had a higher risk (OR=1.90; 95%CI=1.06-3.39). A high consumption of pork remained a rectal cancer risk (OR=1.82; 95%CI=1.05-3.15).

We further estimated the possible effect modifications by *MTHFR* 677 on the effects of major risk factors for rectal cancer (Table 3). The effect modifications were observed between *MTHFR* 677 C/C wild-type and bowel habit; the ORs were 3.24 (95%CI=1.83-5.73) and

25.14 (95%CI=7.00-90.21) for occasional and frequent constipation, respectively, compared with normal defecation. The adjusted OR for *MTHFR* 677 C/T or T/T genotype and having had frequent constipation compared with normal defecation was 11.91 (95%CI=1.20-118.30). The interactions between *MTHFR* 677 and bowel habit were found. The same as for hemorrhoids, the adjusted OR was observed between *MTHFR* 677 C/C wild-type and having had hemorrhoids compared with no hemorrhoids was 4.25 (95%CI=2.33-7.76). The effect modifications were observed between *MTHFR* 677 and a family history of cancer with the risk of rectal cancer. Those who consumed pork at a high level per day and having *MTHFR* 677 C/C wild-type compared with same genotype but low-consumption had a higher risk of rectal cancer (OR=1.78; 95%CI=1.05-3.02).

The joint effects of *MTHFR* 1298 any 'A' genotype and occasional or frequent constipation were statistically significant. The effect modifications were observed between *MTHFR* 1298 and having had hemorrhoids compared with no hemorrhoids with the risk of rectal cancer. Those who consumed pork at a high level per day while having *MTHFR* 1298 A/C or C/C genotype compared with same genotype but low-consumption had a higher risk of rectal cancer.

Discussion

Sriamporn et al. (2007), studied lifestyle-related risk factors for colorectal cancer in Northeast Thailand, but since there may be differences between the natural history of colon and rectal cancer, associations with each site individually are of interest. The present study examines rectal cancer only, and aims to identify associations between these factors and genetic polymorphisms, particularly *MTHFR* genes in relation to folate metabolism, any lifestyle and diet consumption.

The significant risk factors for rectal cancer in the present study were bowel habit (both occasional and frequent constipation), having had hemorrhoids, family history of cancer, and a high level of pork consumption. In the present study, bowel habit was the greatest risk factor for rectal cancer, which was compatible with a previous study on colon cancer in the same area (Promthet et al., 2010). A family history of cancer was the significant risk factor for rectal cancer in this study which is similar to colon cancer risk (Promthet et al., 2010).

Bowel habit (occasional and frequent constipation) was the greatest risk factor for rectal cancer modified by *MTHFR* C/C and C/T or T/T genotypes. Those who had *MTHFR* C/C wild-type had an increased risk for rectal cancer that was modified by having hemorrhoids, and high pork consumption. There were significant interactions between polymorphisms in *MTHFR* 1298 A/A, A/C or C/C genotypes and bowel habit, having hemorrhoids, a family history of cancer, and high level of pork consumption with an increased susceptibility to rectal cancer in the Thai population.

Although none of the *MTHFR* polymorphisms showed any significant effect on rectal cancer risk by genotype alone or between the two polymorphisms, there was a significant increased risk when combined with bowel habit and family history of cancer (P-value for interaction <0.001 and <0.05, respectively). Our finding is similar to the study by Komlósi et al. (2010) who reported that the rectal cancer risk was significantly higher for *MTHFR* CT genotypes (OR=1.4, 95%CI=1.06-1.84) (Komlosi et al., 2010).

The important symptoms that were considered for colorectal cancer diagnosis are rectal bleeding, change in bowel habit, abdominal pain, weight loss, diarrhea and constipation. However, weight loss and rectal bleeding are associated with colorectal cancer (Adelstein et al., 2011). This study confirmed the significant risk of bowel habit, whereas hemorrhoids just were reported in our study. However, bowel habit change correlates to pathogenesis of hemorrhoids that is straining when passing stools (Seow-Choen, 2002) resulting to disrupt of suspensory ligaments of Park at the anal cushion and to prolape of hemorrhoid tissue. In patients with hemorrhoids other abnormalities such as diverticuli, polyps, cancer and vascular lesions can be presented, especially in older patients with age group above 50 years (Koning and Loffeld, 2010). Rectal bleeding is a very common clinical sign and is often caused by hemorrhoids that are the most prevalent anorectal disorder (Janicke and Pundt, 1996) and also is a recognised as early symptom of colorectal (Jones and Kennedy, 1999). Our data found the interaction of these factors with *MTHFR* 677 and 1298 gene polymorphism and showed the risk associated genes. A high level of pork consumption was also found to be the significant risk factors for rectal cancer in the present study. Recently, some study reported that dietary factors such as high intake of carbohydrates and saturated fat may function as initiators in the carcinogenic process (Slattery et al., 2012). The well-done meat also markedly increased risks of colon and rectal cancers but only in individuals with both the rapid NAT2 and CYP1A2 phenotypes (Le Marchand et al., 2002). A number of heterocyclic aromatic amines (HAAs) have been identified in cooked meat at levels that vary according to cooking methods, temperature and duration, and type of meat (Layton et al., 1995). They are the most significant of human exposure and carcinogenic potency (Layton et al., 1995). Patients with constipation might long time expose to several carcinogen. Because our study observed that *MTHFR* 677 and 1298 gene polymorphisms significantly interacted with dietary high level of pork for rectal cancer, this would imply that dietary factors such as high intake of pork in patients with bowel habit change may function as initiators of DNA damage in the carcinogenic process. *MTHFR* polymorphisms are reported to be associated with greater risk for distal colon and rectal cancer than proximal colon tumors (Toffoli et al., 2003; Ulvik et al., 2004), since chromosomal instability occurred greater in the distal than the proximal colon (Lindblom, 2001). These result suggested that folate intake, low methyl donor status and *MTHFR* polymorphisms may play independent roles in the etiology of rectal cancer.

This is the first report showing the relationship between each genotype of *MTHFR* 677, 1298 and the joint effects of both genotypes with the risk of rectal cancer. No studies of this kind have been conducted in Thailand. To

our knowledge, this is the first investigation to detect an association between the common mutations in *MTHFR* on the effects of putative risk factors for rectal cancer in the Thai population.

This study has some limitations. Since ours was a case-control study, recall bias may have occurred during the interview on lifestyle or dietary consumption. Subjects were interviewed focusing on consumption of food items in general but not on dietary folate intake. Further study is needed to pinpoint the dietary folate intake by food frequency questionnaire, and to detect levels of circulating folate and *MTHFR* polymorphisms with the risk of colon/rectal cancers in the Thai population.

In conclusion, this study provided results that support the increased susceptibility to rectal cancer in individuals with *MTHFR* polymorphisms, particularly for individuals who had bowel habits, hemorrhoids, family history of cancer and high pork consumption. Folate may play an important role in mechanisms of rectal cancer initiation/progression by modification of lifestyle or dietary factors. Since genetic factors cannot be modified, prevention by changing lifestyle and dietary pattern is important.

Acknowledgements

The study was supported in part by a grant from The Japanese Ministry of Education, Culture, Sports, Science, and Technology. We are indebted for all the generous assistance from Srinagarind Hospital, Khon Kaen Regional Hospital and Cancer Unit staff at the data collection phase.

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