RESEARCH COMMUNICATION

A Case-Control Study of Colorectal Cancer in Relation to Lifestyle Factors and Genetic Polymorphisms: Design and Conduct of the Fukuoka Colorectal Cancer Study

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Abstract

A case-control study was designed to elucidate roles of dietary and other behavioral influences, in combination with genetic susceptibility factors (genetic polymorphisms), in colorectal carcinogenesis. Both cases and controls were residents in Fukuoka City and three adjacent areas. Cases were patients undergoing surgery for a first diagnosis of colorectal cancer at 8 hospitals in the study area, and controls were randomly selected in the community by frequency-matching with respect to the expected distribution by sex, age (10-year class), and residence . Dietary and other lifestyle factors were ascertained by in-person interview, and venous blood was obtained for genotyping and possible biochemical measurements. The cases were interviewed at each hospital during the period from 2000 to 2003, and controls were surveyed during the period from 2001 to 2002. A total of 840 cases of colorectal cancer and 833 controls were interviewed with participation rates of 80% for the cases and 60% for the controls. Informed consent to genotyping was obtained from 685 cases and 778 controls. Further details of the design and conduct are described with respect to methodological aspects.

Key Words: Colorectal cancer - design - lifestyle factors - genetic polymorphisms - Japan

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Introduction

Colorectal cancer is one of the most common cancers in the world, accounting for nearly 10% of all incident cases (Parkin et al., 2001). In Japan, mortality from colorectal cancer, especially from colon cancer, has increased markedly in the past 50 years (Kono, 1996; Kono and Ahn, 2000), and Japan is currently among the countries with the highest incidence rates of colorectal cancer worldwide (Parkin et al., 2002). Dietary factors have long been implicated in the etiology of the disease, but unequivocal epidemiological evidence remains elusive. The increasing trend of colorectal cancer in Japan has generally been ascribed to the shift to a westernized diet characterized by high intake of fat and meat (Kono, 1996). As extensively reviewed in the report of World Cancer Research Fund (WCRF) and American Institute for Cancer Research (AICR), however, fat intake has been generally found to be unrelated to the risk of colorectal or colon cancer in cohort and case-control studies (WCRF/AICR, 1997). On the other hand, it has been concluded that red meat probably confers increased risk of colorectal cancer (WCRF/AICR, 1997). Although expert panels are in agreement that vegetables are protective against colorectal cancer (WCRF/AICR, 1997; WHO Consensus Panel, 1999), change in vegetable consumption can not explain the increase in colon cancer incidence over time in Japan (Kono and Ahn, 2000). While a low intake of dietary fiber has also attracted much interest in research into the etiology of colorectal cancer, the evidence is not consistent (WCRF/AICR, 1997). The average consumption of dietary fiber earlier in Japan did not differ from the intake in Britain or Denmark with much higher rates of colorectal cancer (Kuratsune et al., 1986).

While several epidemiologic studies have investigated the roles of dietary factors in colorectal carcinogenesis in Japan, none has quantitatively focused on the relation of specific foods and nutrients to the risk of colorectal cancer. We therefore are now conducting a case-control study to investigate comprehensively the relation of lifestyle and genetic factors to colorectal cancer in Fukuoka, Japan. Our

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primary aim was to clarify roles for meat, fat, vegetables, and fiber in the development of colorectal cancer, but the study was also designed to address associations with other dietary and non-dietary factors as well. Possible protective factors unique to the Japanese diet such as green tea and soy foods are of particular interest (Kono, 1992; Yamane et al., 1996; Toyomura and Kono, 2002). Other targets include genetic polymorphisms linked with the metabolism of carcinogens or protective compounds (Vineis et al., 1999) and their interactions with dietary factors. This paper describes methodological aspects in the design and conduct of the study.

Study Design

Overview

This case-control study was designed to elucidate roles of dietary and other behavioral factors and genetic susceptibility factors (genetic polymorphisms) in colorectal carcinogenesis. Both cases and controls were residents in Fukuoka City and three adjacent areas. Cases were patients undergoing surgery for a first diagnosis of colorectal cancer, and controls were randomly selected from the community by frequency-matching with respect to sex, age (10-year class), and residence (Fukuoka City or the adjacent areas). Dietary and other lifestyle factors were ascertained by in-person interview, and venous blood was obtained for genotyping and possible biochemical measurements.

Size of the Study

The study aimed to recruit 500 cases of colorectal cancer and 1,000 controls. In determining the sample size, we first assumed detection of at least a 2-fold difference in the odds ratios for the highest versus lowest quartile of an exposure under study (such as fat and meat intake) at the two-sided significance level of 0.05 with a detection power of 80%. It was then calculated that at least 272 cases and 272 controls were required; the estimated size was 136 in each group when the prevalence of the exposure was 50%, and this number was doubled with allowance for the middle 50%, i.e. the second and third quartiles. With the prevalence of a factor under study at 5% in the population, the required numbers of cases and controls were estimated to be 515 each. Based on these preliminary estimations, we considered that 500 cases of colorectal cancer and 1,000 controls would be necessary for investigation of associations with rare genetic polymorphisms and interactions. A study of 500 cases and 1,000 controls corresponds to one with 660 cases and 660 controls with the same significance level and power.

Selection of Cases

Cases were a consecutive series of patients with histologically confirmed colorectal adenocarcinomas who were admitted to two university and affiliated hospitals for the first-time surgical treatment. Other eligibility criteria included the following characteristics: age of 20-74 years at the time of diagnosis; residence in Fukuoka City or three adjacent areas (Chikushi, Kasuya, and Itoshima); no prior history of partial or total removal of the colorectum, familial adenomatous polyposis (FAP), or inflammatory bowel disease; and mental and physical competence to give informed consent and to complete the interview.

Selection of Controls

Eligibility criteria for controls were the same as described for the cases except for two conditions, i.e. having no diagnosis of colorectal cancer and age of 20-74 years at the time of selection. It was decided a priori to select 1,500 persons as control candidates by two-stage random sampling, with two thirds of them expected to actually participate in the study. Numbers of control candidates by sex and 10-year age class were determined in proportion to numbers of incident cases of colorectal cancer by sex and 10-year age class in the study area, which were estimated from incidence rates in the Osaka cancer registry during the period 1988 to 1992 (Parkin et al., 1997). The first step was a random selection of 15 small areas which roughly corresponded to primary-school zones, and residents were randomly selected in each of these using the municipal resident registry, with allowance for proportions of residents for each small area by sex and 10-year age class.

Lifestyle Questionnaire

The questionnaire was developed for research nurses to perform a uniform interview with cases and controls regarding multifaceted lifestyle factors. The questionnaire used for the cases slightly differed from that of the controls in that the former included questions regarding the symptoms or screening leading to the diagnosis of colorectal cancer. The cases were asked whether symptoms or screening were events leading to the diagnosis, and if so the duration in months from the onset of symptoms or the time of the screening to the interview. The cases were carefully asked to answer to each question about their lifestyle habits and physical conditions in the period before the onset of the symptoms or the screening. Most of the questions were closed-ended, and open-ended questions were used for quantitative measures such as amount of alcohol and time spent in physical activities. Demographic information was confined to sex, date of birth, and area of residence.

Anthropometric questions inquired about height (cm), recent body weight (kg), and body weight 10 years before symptoms or screening in the cases and before the interview in the controls. Recorded heights and body weights were also obtained if they were available.

Questions on physical activities elicited type of job, activities in commuting and housework shopping, and leisure-time activities 5 years previously. As regards type of job, 5 options were prepared: sedentary or standing work (clerical work, taxi driving, housework, etc), work with walking (delivery by walking, patrolling on foot, etc), labor work (construction work, agricultural work, load transport, etc), hard labor work (digging or chopping with heavy tools, carrying heavy loads, etc), and no job (including students).

Weekly minutes spent in walking, bicycling, and jogging were each ascertained regarding commuting and housework shopping on average in the year. Regular leisure-time activities, on average over one year, were ascertained with regularity defined as at least once per week. For up to three activities, type, numbers of months and of days per week that individuals participated in each, and minutes of participation per occasion were reported. Intensity of each physical activity was determined in terms of metabolic equivalents (METs) on the basis of the published compilation of physical activities (Ainsworth et al., 1993), and MET-hours can be calculated as indices of non-occupational physical activities.

With regard to the smoking habit, individuals were first asked whether they had ever smoked cigarettes everyday for one year or longer. Then they were asked about the current status (before the symptom or screening in the cases). Age of starting smoking and that of quitting smoking (for past smokers) were ascertained, along with years of smoking and numbers of cigarettes smoked per day for each decade of age from the second to eighth decade.

Alcohol use until 5 years prior to the interview was elicited with alcohol use defined as drinking alcoholic beverages at least once per week over the period of one year or longer. Then individuals answered open-ended questions regarding the frequency of consumption (number of days per week) and amount of alcohol consumed on the day of alcohol drinking, on average over the year at the time of 5 years prior to the interview. The amount of alcohol was expressed by the conventional unit; one go (180 ml) of sake, one large bottle (633 ml) of beer, and half a go (90 ml) of shochu were each expressed as one unit; and one drink (30 ml) of whisky or brandy and one glass (100 ml) of wine were each converted to half unit. A supplementary question was asked about the psycho-social circumstances of drinking alcohol with four precoded answers: “for self-enjoyment or social events”, “to pacify unhappy feeling”, “unwillingly but for keeping acquaintance”, and “for self-appreciation”.

Prior histories of physician’s diagnosis and surgeries included diabetes mellitus, gallstones, cholecystectomy, gastrectomy, colorectal polyp, colorectal polypectomy, large-bowel resection, appendicectomy, angina pectoris, myocardial infarction, cerebral infarction, femoral fracture, and other diseases leading to hospital admission. The time of diagnosis or surgery was elicited, and the related surgical scars were confirmed.

Use of vitamins, analgesics, and cholesterol-lowering drugs were recorded if these had ever been used at least once per week for a period of 6 months or longer. Activity of daily living was ascertained with classification of four categories (perfect independence, independence at home with need of help on the occasion of going outside, support needed at home, and bedridden). Also determined were parental history of colorectal cancer and the age of development of the disease.

Ten questions as to bowel habits were posed in a closed-ended fashion: frequency of bowel movement, frequency of feeling constipation, use of purgatives, regularity of bowel movement, time required for defecation, nature of stool, sense of residual stool, sense of bowel distension, frequency of fecal incontinence, and frequency of stool staining on the underwear.

Numbers of children, brothers, and sisters were reported. Women reported age of menarche, age of menopause, number of parity, and years after the last pregnancy. In addition to the interview on lifestyle factors, individuals were asked to complete a self-administered questionnaire containing 45 closed-ended questions regarding psychosocial stress and personality (Nagano et al., 2001).

Dietary Assessment

A PC-software package was developed for dietary assessment with support from an external laboratory (Core Create Systems, Kitakyushu). A total of 148 items of foods and beverages were selected with reference to the results from the national nutrition survey (Japan Ministry of Health, Labour and Welfare, 1999) and dietary questionnaires developed elsewhere in Japan (Tsubono et al., 1996; Tokudome et al., 1998). Frequency of consumption and portion size were elicited. Typical dishes were shown on the display, together with average portion sizes. Options for serving size were 0.5, 1, 1.5, and 2 with the displayed size as referent (average size) for most of the food items; more detailed options were prepared for the portion size of rice. Supplementary questions inquired about consumption of fatty portions of beef/pork and skin of chicken at table, consumption of soup for noodle dishes, rank of consumption as to 5 types of hot-pot dish cooked at the table, consumption pattern of vegetables and meat in hot-pot dishes, and use of sugar for coffee and black tea.

Ethical Considerations

The study protocol was approved by the ethical committees of Kyushu University and of all but two of the participating hospitals; the two hospitals had no ethical committee at the time of the survey, and approval was obtained from the director of each hospital. The objectives and overall design of the study were explained verbally to each potential case or control subject with the explanatory document shown, and a consent form was presented to him or her. Consent was sought for participation in the interview, genotyping, biochemical measurement, and a self-administered questionnaire on psychosocial stress and personality separately. The interview was started after he/she signed the consent form. A carbon copy of the signed form together with the explanatory document was handed to the participant.

Conduct of the Survey

Overview

A total of 840 cases of colorectal cancer and 833 controls were interviewed with participation rates of 80% and 60%, respectively. Gender, age, residence area, and consent to

genotyping are summarized in Table 1. Six research nurses were in charge of the interviews of both cases and controls, and one research clerk was involved in the recruitment of controls and monitored the progress of the survey.

Survey of Cases

The cases were interviewed in the wards of each hospital during admission. Research nurses visited each hospital weekly, and determined eligibility of cases by referring to admission logs and medical records. They then contacted each eligible patient with permission from the attending doctor, and interviewed him/her if written informed consent was given. A co-investigator at each hospital was in charge of supervising the survey of the cases. The survey started at two university hospitals and three affiliated hospitals in September or October in the year 2000 and at another three affiliated hospitals in May in the year 2001. The survey ended in December, 2003. At one university hospital, the survey at one of the two wards accommodating fewer surgical cases of colorectal cancer was delayed until December, 2002.

In the consecutive series of potentially eligible 1099 cases, 19 were found to be mentally incompetent; 23 had no histological diagnosis of colorectal adenocarcinomas; 3 were found to have a history of large-bowel resection after the interview; and one had FAP. After exclusion of these 46 cases, 840 (80%) of the 1053 eligible cases participated in the interview, and 685 (82%) gave informed consent to genotyping. Reasons for not participating in the study were patient's refusal (n = 115), physician's refusal (n = 46), and missing contact (n = 52). Five patients had previously been interviewed as controls in this study, and one of them had refused participation. The other four were not approached intentionally. Participation rates by sex and 10-year age class are shown in Table 2. Elderly patients were less likely to take part than younger adults, and the overall participation rate was slightly better in men than in women. It was later noticed that one interviewed case was aged 75 years at diagnosis, and this case was included in the study.

The occurrence of a relevant symptom resulted in the diagnosis of colorectal cancer for 556 cases, and screening was the event leading to diagnosis in 284 men. The duration from the onset of a symptom or screening until the interview ranged 0 to 36 months with a median of 2 months; the duration exceeded 12 months in 15 cases. The interview was conducted before the surgery in 551 cases (66%). Numbers of interviewed cases according to the location of the colorectal cancer were as follows: proximal colon 191, distal colon 279, rectum 354, and multiple sites 16. Cecum, ascending colon, and transverse colon were combined as proximal colon, and distal colon included descending and sigmoid segments.

Table 1. Overview of the Survey of Cases and Controls

	Cases	Controls
Number of subjects interviewed	840	833
Participation rate	80%	60%
Numbers of men/women	501/339	515/318
Mean age (range)	61 (27-75)[a]	59 (22-74)[b]
Residence in Fukuoka City	61%	65%
Length (min) of interview, median (range)	50 (27-105)	52 (25-133)
Number of consent to genotyping	685	778

[a] Age at diagnosis. One interviewed case aged 75 years was included.
[b]Age at random sampling.

Survey of Controls

In the random selection of 15 small areas in the study area, primary-school zones were preliminary reviewed with respect to location and population size. After merging sparse-population school zones and slight modification with allowance for administrative zones, 145 primary-school zones in Fukuoka City and 93 primary-school zones in the adjacent areas were restructured to 115 and 63 small areas, respectively. Population at the age of 20-74 years was twice larger in Fukuoka City than in the adjacent three areas, and thus we decided to select 10 small areas in Fukuoka City and 5 small areas in the adjacent areas and 1,000 residents in the former and 500 residents in the latter. Numbers of residents by sex and 10-year age class in each small area were determined on the basis of proportions of residents among those in the total of 10 small areas in Fukuoka City or the total 5 small areas in the adjacent areas by sex and 10-year age class. The number of residents selected in the small areas ranged from 66 to 143.

Recruitment and survey were initiated by a letter of invitation, sent to each control candidate, and a telephone call was made if the candidate was listed in the telephone

Table 2. Participation of Cases in the Interview Survey by Sex and Age Class

	Men		Women		Both	
Age (yr)*	No.#	%	No.	%	No.	%
20-29	-	-	2/3	67	2/3	67
30-39	8/9	89	7/7	100	15/16	94
40-49	44/52	85	35/38	92	79/90	88
50-59	147/173	85	111/137	81	258/310	83
60-69	204/249	82	117/161	73	321/410	78
70-75	98/131	75	67/93	72	165/224	74
Total	501/614	82	339/439	77	840/1053	80

* Age at diagnosis. # Numbers of participants/eligible persons.

directory. Two letters of invitation were additionally mailed to non-respondents. After completion of the survey in the 15th small area, a letter requesting participation was sent to 193 persons who had not responded to the third mail and 375 persons who had once refused the participation without hostility or explicit reluctance. In response to this final invitation, 10 (5%) of the former and 23 (6%) of the latter participated in the study.

The mail invitation revealed that a proportion of the control candidates were actually ineligible for the study for the following reasons: death (n = 7), migration from the study area (n = 22), undelivered mail (n = 44), history of large-bowel resection (n = 21), and mental incompetence (n = 19). Further, as mentioned above, there were participants who were diagnosed as having colorectal cancer after the survey (n = 5). After exclusion of these 118 persons, 833 (60%) of the 1382 eligible candidates were participants in the study. Overall, there was no response to the invitations by 158, and 391 persons refused. Participation rates by sex and 10-year age class are shown in Table 3. Women, especially in their 40s and 70s, were less willing to participate in the study than men. Consent to genotyping was obtained from 778 (93%) of the interviewed controls.

The survey was performed during the period from January 2001 to December 2002. The interviews of controls were conducted at community halls (n = 615), collaborative clinics (n = 170), the Department of Preventive Medicine at Kyushu University (n = 22), home (n = 14), work place (n = 8), and hospitals where control subjects were admitted (n = 4). An investigator physician attended to the survey at community halls, work place, and home. When the survey was done at a collaborative clinic, supervision was by a physician at the clinic. A gratuity of 5,000 Japanese yen was paid to each participant.

Validation Study of Dietary Assessment

A total of 60 men and women aged 40-64 years were asked to participate in a validation study. They were controls who participated in the survey in two small areas conducted from April to July in the year 2001. Of the 60 persons to whom requests were made, 36 agreed to participate in the validation study. They were asked to weigh (or record when weighing was not possible) foods and beverages consumed each day over one week each in four seasons (July-August 2001, October-November 2001, January-February 2002, and April-May 2002). In the course of the study, however, 8 persons withdrew, and only 28 persons completed the dietary record. In July, 2002, the interview was repeated using the same questionnaire and the PC software for dietary assessment. Results of the validation study will be published elsewhere.

Storage of Blood Samples

A vacuum tube containing EDTA-sodium was used to draw 5 ml of venous blood. Blood samples were stored temporarily in a refrigerator, and were centrifuged to separate plasma and buffy coat within the day of collection. Two aliquots of 1 ml plasma and buffy coat were stored at -80 ^{o}C. DNA was extracted from the buffy coat by using a commercial kit (QIAGEN GmbH, Hilden), and was stored at -40 ^{o}C. DNA concentrations ranged approximately from 50 to 150 ng/μl.

Informed Consent

Name and residence address of each participant were recorded on the signed form of informed consent, but not on the questionnaire. Only sex and date of birth were recorded. All of the signed forms of informed consent were transferred to the custody of the Kyushu University official in charge of protection of personal identification information. The questionnaire and blood sample were labeled with a unique anonymous number, which was recorded on the informed consent form as well.

Discussion

This is probably the fourth largest case-control study of colorectal cancer using community controls in the world. The larger investigations were a population-based study of 1993 cases and 2410 controls in the mainland of the United States (Slattery et al., 1997), 1192 pairs of cases and controls in Hawaii (Le Marchand et al., 1997), and 931 cases and 1552 controls in Shanghai (Chiu et al., 2003). While over 90% of the population was white in the study by Slattery et al (1997), the population was very diverse in ethnicity in Hawaii (Le Marchand et al., 1997). In the latter, Japanese,

Table 3. Participation of Controls in the Interview Survey by Sex and Age Class

	Men		Women		Both	
Age (yr)[a]	No[b]	%	No	%	No	%
20-29	2/8	25	6/9	67	8/17	47
30-39	21/30	70	17/29	59	38/59	64
40-49	62/102	61	35/73	48	97/175	55
50-59	167/272	61	79/144	55	246/416	59
60-69	180/269	67	131/216	61	311/485	64
70-74	83/132	63	50/98	51	133/230	58
Total	515/813	63	318/569	56	833/1382	60

[a] Age at random sampling. [b]Numbers of participants/eligible persons.

who were the majority of the population, numbered 598 pairs of cases and controls. Numbers of cases and controls decreased substantially in the analysis of genetic polymorphisms; approximately 1500 cases and 1800 controls were included in a study by Slattery et al (1999), and around 500 cases and 600 controls remained in the study in Hawaii (Le Marchand et al., 2002). The size of study is particularly important in investigating the role of rare genotypes in the gene-environment or gene-gene interaction. High participation rates of both cases and controls are required in case-controls studies to reduce selection bias. In the present study, the participation rate was not as high in the controls as attained for the cases. It is generally difficult to attain a high participation rate for community controls. In the recruitment of controls in the present study, the invitation was repeated four times when responses were not obtained. The net participation rate, which was calculated after exclusion of ineligible persons, was 45.3% at the first invitation, and the rate increased by 10.1% after the second invitation, and by 1.3% after the third invitation. Together with the fourth mailing to non-respondents, we once repeated the invitation to those who had refused to participate. This final attempt further increased the participation by 2.4%. The invitation repeated after the second was less efficient, but the participation rate of 60% was only achieved with the third and fourth attempts. Although a higher participation rate would of course be desirable, a rate of 60% is considered to be acceptable (Olson, 2001). The participation rates of controls were 64% in the above-mentioned largest study in the United States (Slattery et al., 1997), 71% in Hawaii (Le Marchand et al., 1997), and 85% in Shanghai (Chiu et al., 2003).

While we attained a fairly good participation of cases, consideration is needed with respect to their representativeness. The survey of cases was not performed at all hospitals in the study area where surgical treatment for colorectal cancer is performed. In particular, there are two other large hospitals located in the same area. However, it is unlikely that the patients included in the present study did differ from those at the other hospitals. We confined the eligible cases to those undergoing surgery for the purpose of mitigating psychological stress on both patients and interviewers. This was probably one reason for the high participation, but we missed some cases with advanced colorectal cancer. The interview of the cases during the admission may have been another reason for the high participation of 80%, and this procedure had another methodological advantage of shortening the period from disease onset to interview. Population-based case-control studies usually rely on information from cancer registries, and the interview is delayed much longer after the onset of the disease.

To our knowledge, 8 case-control studies have hitherto addressed the relation between food consumption and colorectal cancer in Japan. In three (Kato et al., 1990; Hoshiyama et al., 1993; Nishi et al., 1997), controls were recruited in the community and the participation rate was reported in only two studies (Kato et al., 1990; Hoshiyama et al., 1993). The earliest study was that conducted by Wynder et al (1969), in which 107 cases and 307 controls were interviewed at two hospitals in Tokyo. Cases of colon cancer, but not of rectal cancer, tended to have a diet lower in rice and higher in fruit and milk. Kondo (1975) carried out an interview survey on 393 cases and 786 controls at three hospitals in Nagoya in the period 1967-1973. Using a questionnaire containing 107 food and beverage items, the author reported more frequent consumption of eggs and chicken in colon cancer patients and a higher frequency of foods high in carbohydrate and low in animal protein in cases of rectal cancer. Haenszel et al (1980) surveyed 588 colorectal cancer patients and 1,176 hospital controls in Hiroshima, Aichi, and Miyagi Prefectures. They included over 100 food items in the questionnaire used for interview, but found no material association with most of the foods and beverages except for inverse associations with a few vegetables. Watanabe et al (1984) conducted a study of 203 matched-pairs at five hospitals in Kyoto, Shiga, and Hyogo Prefectures in the period 1977-1983, and noted a suggestive protective association with tofu and beans combined for rectal cancer. Tajima and Tominaga (1985) conducted a study of 93 cases and 186 controls at Aichi Cancer Center Hospital in the period 1981-1983. High consumption of chicken, pork, and beef were each associated with statistically significant or non-significant increase in the risk of colon and rectal cancer. Kato et al (1990) used a self-administered questionnaire, which elicited the consumption of 25 foods, in a study of 221 cases at Aichi Cancer Center Hospital during the period 1986-1990 and 586 controls randomly selected from telephone directories. The controls were respondents to a mailed questionnaire, and the response rate was 91%. Daily intake of meat was related to decreased risks of colon and rectal cancer. Hoshiyama et al (1993) carried out a study of 181 cases admitted to Saitama Cancer Center Hospital during the period of 1984-1990 and 653 community controls. The controls were 27% of residents randomly selected in the vicinity of the Hospital using the electoral rolls. Of 24 food items under study, seaweed consumption was inversely associated with both colon and rectal cancer, and pickled vegetables showed a positive association with rectal cancer. In the study reported by Nishi et al (1997), 330 cases and 660 community controls were interviewed in Hokkaido during the period 1987-1990, with controls randomly selected from telephone directories. Of 19 food items under study, visceral meat and ham/sausage were each associated with increased risks of colon and rectal cancer.

While several cohort studies have been documented regarding the relation of smoking or alcohol use to colorectal cancer in Japan (Kono et al., 1987; Hirayama, 1990; Shimizu et al., 2003; Otani et al., 2003), prospective data regarding diet and colorectal cancer in Japan are only available from the Hirayama cohort study which ascertained consumption frequencies for 7 food items (Hirayama, 1990).

Epidemiological studies in recent years have added to

evidence that calcium and folate are protective in the development of colorectal cancer, whereas evidence for protective associations with these factors was judged as insufficient in the report of the WCRF/AICR (1997). Randomized trials consistently observed that calcium supplementation resulted in a measurable reduction in the recurrence of colorectal adenomas, a well established precursor lesion for the colorectal cancer (Baron et al., 1999; Bonithon-Kopp et al., 2000). Further a pooled analysis of prospective data in Europe and the United States showed a deceased risk of colorectal cancer associated with calcium intake (Cho et al., 2004). Studies of a genetic polymorphism of methylenetetrahydrofolate reductase, a key enzyme in folate metabolism, have strengthened evidence for a protective role for folate in the occurrence of colorectal cancer (Matsuo et al., 2002; Sharp and Little, 2004). The pros and cons have been argued regarding red meat and colorectal cancer since the report of WCRF/AICR (1997). Hill (2001) argued against the conclusion in the WCRF/AICR report. A recent cohort study failed to find an increased risk of colon cancer associated with meat as a whole or red meat (Flood et al., 2003), whereas a meta-analysis showed a modest increase in the risk of colorectal cancer associated with red and processed meat (Norat et al., 2002). On the other hand, in an ecological study in Japan (Kono, 2004), colon cancer incidence rates were strongly correlated with meat consumption approximately 20 years earlier. Studies of metabolic polymorphisms suggested that increased risk of colorectal cancer associated with red meat consumption might be due to exposure to nitrosamines (Le Marchand et al., 2002) and heterocyclic amines (Le Marchand et al., 2001). Heme-iron intake might also have been responsible for the association between red meat and colorectal cancer as shown in another study (Lee et al., 2004).

Finally, 685 cases and 778 controls gave consent to genotyping in the present study. Studies on genetic polymorphisms of known functions not only shed light on mechanisms of environmental factors increasing or decreasing the risk of colorectal cancer, but also have the potential to provide decisive conclusions regarding inconsistent associations with environmental factors in case-control and cohort studies. Our relatively large number of colorectal cancer cases will enable us to examine lifestyle factors and genetic polymorphisms according to subsites in the colorectum. Etiological factors may vary not only between colon and rectal cancer but also between segments of the colon. Recent studies have shown different molecular alterations between proximal and distal sites of the colorectum (Breivik et al., 1997; Elsaleh et al., 2000).

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