Association between genetic polymorphisms of the base excision repair gene *MUTYH* and increased colorectal cancer risk in a Japanese population

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The *MUTYH* gene encodes a DNA glycosylase that can initiate the base excision repair pathway and prevent G:C > T:A transversion by excising adenine mispaired with 8-hydroxyguanine. Biallelic germline mutations of *MUTYH* have been shown to predict familial and sporadic multiple colorectal adenomas and carcinomas, however, whether there is an association between single nucleotide polymorphisms (SNPs) of *MUTYH* and sporadic colorectal cancer (CRC) risk has remained unclear. In this study we investigated four *MUTYH* SNPs, IVS1+11C > T, IVS6+35G > A, IVS10–2A > G, and 972G > C (Gln324His), for an association with increased CRC risk in a population-based series of 685 CRC patients and 778 control subjects from Kyushu, Japan. A statistically significant association was demonstrated between IVS1+11T and increased CRC risk (odds ratio [OR]: 1.43; 95% confidence interval [CI]: 1.012–2.030; $P = 0.042$) and one of the five haplotypes based on the four SNPs, the IVS1+11T – IVS6+35G – IVS10–2A – 972C (TGAC) haplotype containing IVS1+11T, was demonstrated to be associated with increased CRC risk (OR, 1.43; 95% CI, 1.005–2.029; $P = 0.046$). Subsite-specific analysis showed that the TGAC haplotype was statistically significantly ($P = 0.013$) associated with an increased risk of distal colon, but not proximal colon or rectal cancer. Furthermore, IVS1+11C > T was found to be in complete linkage disequilibrium with –280G > A and 1389G > C (Thr463Thr). The results indicated that Japanese individuals with –280A/IVS1+11T/1389C genotypes or the TGAC haplotype are susceptible to CRC. (*Cancer Sci* 2008; 99: 355–360)

Intracellular DNA is at risk of damage by reactive oxygen species (ROS) generated by normal metabolism and environmental exposure, and 8-hydroxyguanine (8-ohG) is one of the products induced by ROS damage and is known to be a mutagenic lesion.[1,2] The base excision repair (BER) pathway plays an important role in repairing oxidative-damage-induced mutations, and the *MUTYH* gene encodes the glycosylase capable of initiating the BER pathway by catalyzing the removal of adenine residues mispaired with 8-ohG.[3–5] It has been indicated that defects in the BER pathway may contribute to tumorigenesis by increasing mutation frequency in oncogenes and tumor suppressor genes.[6] In fact, it has been reported that some cases of autosomal recessive inherited multiple colorectal adenomatous polyposis and carcinoma with an increased frequency of somatic G:C > T:A mutations in *APC* are attributable to biallelic germline mutations in the *MUTYH* gene.[7–10] The disease-causing mutations, Y165C, G382D, 466delE, E466X, and Y90X have been reported in Caucasians, Indian, Pakistani and other ethnic groups.[8,9,11,12] The frequencies of Y165C and G382D have been investigated in several colorectal cancer (CRC) case-control studies, and monoallelic carriers of these variants were found in 0.0–2.6% of the cases and 0.0–2.1% of the controls and biallelic carriers of these variants were found in 0.0–0.8% of the cases and 0% of the controls, respectively.[13–18] However, neither of these two variants has ever been detected in East Asians, including Japanese,[19–22] suggesting that they are ethnicity-specific alleles. Based on the above findings, we hypothesized that *MUTYH* variants other than Y165C and G382D act as low-penetrance susceptibility alleles in Japanese CRC, similar to a situation previously reported for the *APC* and *CHEK2* gene variants.[23,24]

We conducted a CRC case-control study to evaluate the significance of *MUTYH* variants in a Japanese population. In the single-nucleotide polymorphisms (SNPs) reported in the Japanese population,[19,20] four SNPs (IVS1+11C > T, IVS6+35G > A, IVS10–2A > G and 972G > C [Gln324His]), were selected, and all 685 cases and 778 matched controls were genotyped to detect these four SNPs. Statistically significant association was found between the IVS1+11C > T SNP and increased CRC risk in the Japanese population. A haplotye-based association study was also carried out, and a statistically significant association was found between the IVS1+11T – IVS6+35G – IVS10–2A – 972C (TGAC) haplotype containing the IVS1+11T allele and CRC risk. In the subsite-specific analysis, the IVS1+11C > T SNP was detected to be nearly statistically significantly associated and the TGAC haplotype was found to be statistically significantly associated with an increased risk of distal colon, but not proximal colon or rectal cancer. We also found that a novel –280G > A SNP in the 5′ flanking region of *MUTYH* and a previously reported 1389G > C (Thr463Thr) SNP were both in complete linkage disequilibrium with the IVS1+11C > T. Our results suggest that the –280A/IVS1+11T/1389C or the TGAC haplotype of *MUTYH* may be novel CRC susceptibility alleles.

Materials and Methods

Specimens. Blood specimens from 685 CRC cases and 778 controls were collected in a previous study. DNA was extracted from these specimens and written informed consent was obtained

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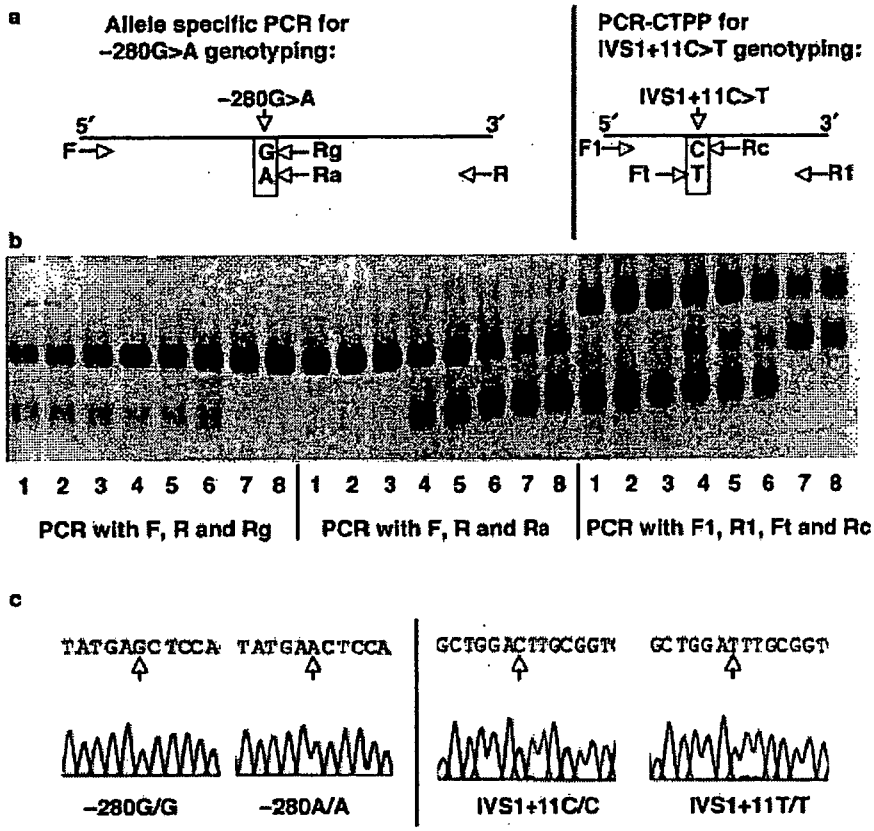

Fig. 1. Genotyping of the –280G > A and IVS1+11C > T single nucleotide polymorphisms (SNPs) of the *MUTYH* gene. (a) The schematic diagrams of the allele-specific polymerase chain reaction (PCR) used to genotype the –280G > A SNP (left) and the PCR with confronting two-pair primers (PCR-CTPP) used to genotype the IVS1+11C > T SNP (right). PCR primers are indicated by the horizontal arrows, and F and R mean forward primer and reverse primer, respectively. The location of each SNP is indicated by a vertical arrow. (b) Agarose gel electrophoresis of the PCR products. Eight samples, three from homozygous carriers of the wild-type allele (No. 1–3), three from heterozygous (No. 4–6) and two from homozygous (No. 7 and 8) carriers of the variation, were genotyped for –280G > A (left and middle) and IVS1+11C > T (right). (c) Sequence electropherograms of the region containing the –280G/G and A/A (left two) and IVS1+11C/C and T/T (right two). The positions of the SNPs are indicated by vertical arrows.

from each individual patient.[25] The characteristics of the cases and controls have been described previously.[25–28] In brief, the cases were composed of a consecutive series of patients with histologically-confirmed incident colorectal adenocarcinomas, and controls were composed of individuals that had no diagnosis of CRC. Other eligibility criteria were as follows: age 20–74 years at the time of diagnosis for the cases or at the time of selection for the controls, residents of the study area (Fukuoka City and three adjacent areas), no prior history of partial or total removal of the colorectum, familial adenomatous polyposis or inflammatory bowel disease, and mental competence to give informed consent and participate in the interview. The number of control candidates by gender and 10-year age class was determined in accordance to the expected sex- and age-specific number of incident cases of colorectal cancer. For the reverse transcriptase-polymerase chain reaction (RT-PCR) experiment, total RNA was extracted from the non-cancerous colorectal mucosa of six CRC patients and converted to cDNA, as described previously.[19] This study was approved by the Institutional Review Board (IRB) of Hamamatsu University School of Medicine (12–14, 18-4).

Target SNPs and genotyping. The six SNPs genotyped in this study were as follows: IVS1+11C > T (rs2275602), IVS6+35G > A (rs3219487), IVS10–2A > G (5′-flanking sequence: 5′-CAC TCA ACC CTG TGC CTC TC-3′; 3′-flanking sequence: 5′-GGT GGA GCA GGA ACA GCT CT-3′), 972G > C (Gln324His) (rs3219489), G382D (rs36053993), and –280G > A (5′-flanking sequence: 5′-ATT ACT ACT AAC CGT TAT GA-3′; 3′-flanking sequence: 5′-CTC CAG ACT ACA TCT CCC GC-3′). The IVS10–2A > G and –280G > A had not been presented in the SNP database (dbSNP) of the National Center for Biotechnology Information (NCBI) Entrez system. Genotyping of the four target SNPs, namely, IVS1+11C > T, IVS6+35G > A, IVS10–2A > G and 972G > C (Gln324His), was carried out by PCR with confronting two-pair primers (PCR-CTPP), as described previously (Fig. 1a,b),[19] and genotyping of the G382D SNP was carried out by PCR-restriction fragment length polymorphism (PCR-RFLP). Genotyping of the –280G > A SNP was carried out by two independent allelic-specific PCRs (Fig. 1a,b). The PCR primers used were: IVS1+11C > T SNP: F1 (5′-AAC TAT GAG CCC GAG GCC TTC C-3′), R1 (5′-CAG CAG AAC ACG GAG GCC C-3′), F2 (5′-AGT CGT CTG TGG GTA CGC TGG AT-3′), and R2 (5′-CCA GGA GAC GGA CCG CAA G-3′); IVS6+35G > A: F1 (5′-CCA GTG TGG GTC TCA GAG G-3′), R1 (5′-CCC TAG CTC CTC TAC CAC CTG-3′), F2 (5′-CTA GGG TAG GGG AAA TAG GAA CA-3′), and R2 (5′-CAC CCG TCA GTC CCT CTA TC-3′); IVS10–2A > G SNP, those described previously,[19] 972G > C (Gln324His) SNP: F1 (5′-CCT GTC GGG CAG TCC TGA CG-3′), R1 (5′-CGC TGA AGC TGC TCT GAG GGC-3′), F2 (5′-CCC AGC TCC CAA CAC TGG ACA C-3′), and R2 (5′-GAG GCA GGC ACA GGT GGC AC-3′); G382D SNP: F (5′-GCC CAA ATT CTG CTG GTG C-3′) and R (5′-GCC CAA CGC TGT AGT TCC TG-3′); –280G > A SNP, F (5′-TAC TGT TCT CAT GGT GCC CC-3′), R (5′-GCC TCG GGC TCA TAG TTC TAG-3′), Ra (5′-GCG GGA GAT GTA GTC TGG AGT-3′), and Rg (5′-CGG GAG ATG TAG TCT GGA GC-3′). PCR products were fractionated by electrophoresis on a 2.0% agarose gel and stained with ethidium bromide. All the cases and controls were genotyped for all of the above SNPs.

Statistical analysis. χ^2 tests were used for deviation from the Hardy–Weinberg equilibrium (HWE) among the controls, and the significance level was set at 0.05. Associations between *MUTYH* genotypes or haplotypes and risk of CRC were assessed by calculating the odds ratio (OR) and 95% confidence interval (CI). SAS version 8.2 software (SAS institute, Inc., Cary, NC, USA) was used to carry out the statistical analysis. A *P*-value less than 0.05 was accepted as statistically significant in all cases. Adjustment for multiple testing was performed using false discovery rate (FDR) principle.[29] Haplotypes were inferred by

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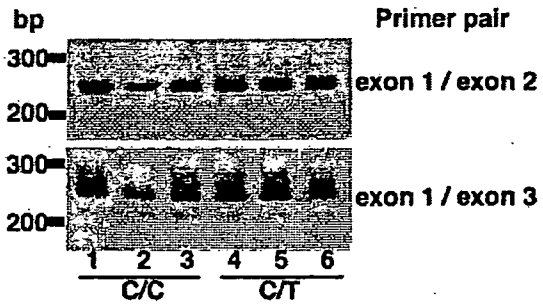

Fig. 2. Reverse transcription-polymerase chain reaction (RT-PCR) analysis. RT-PCR was carried out with a set of primers located at exon 1 and exon 2 (upper panel) and a set of primers located at exon 1 and exon 3 (lower panel). cDNAs from three homozygous carriers of the wild-type allele (lanes 1–3) and three heterozygous carriers of the variation (lanes 4–6) were used as the templates.

the expectation maximization algorithm with the SNPAlyze Version 5.0 software (DYNACOM, Yokohama, Japan). Five haplotypes with a frequency of greater than 1% were selected for further statistical analysis. The linkage disequilibrium analysis of the haplotypes was carried out using the SNPAlyze Version 5.0 software (DYNACOM).

RT-PCR analysis. Reverse transcription-polymerase chain reaction was carried out for the IVS1+11C/C and C/T genotype, respectively, with 1 μL of each cDNA prepared from the non-cancerous colorectal mucosa of six CRC patients. The two primer pairs shown in Fig. 2 were used: one pair was composed of a forward primer in exon 1 and a reverse primer in exon 2, and the other pair was composed of the same forward primer in exon 1 and a reverse primer in exon 3. The sequences of the primer sets are available on request.

Results

Target SNP selection. Among the variants registered in the dbSNPs of the NCBI Entrez system, there were six *MUTYH* SNPs, namely, IVS1+11C > T (rs2275602), IVS1+1841G > A (rs3219472), IVS1+3221T > G (rs3219476), IVS6+35G > A (rs3219487), IVS14–40G > C (rs3219493) and 972G > C (Gln324His) (rs3219489), that have been detected in the Japanese population. Among the SNPs reported in previous publications,[19,20] five *MUTYH* SNPs, namely, IVS1+11C > T (rs2275602), IVS6+35G > A (rs3219487), IVS10–2A > G (5′-flanking sequence: 5′-CAC TCA ACC CTG TGC CTC TC-3′; 3′-flanking sequence: 5′-GGT GGA GCA GGA ACA GCT CT-3′), 972G > C (Gln324His) (rs3219489), and 1389G > C (Thr463Thr) (5′-flanking sequence: 5′-CCA GGT GCT CGC TGG CTG AC-3′; 3′-flanking sequence: 5′-CAG GAG GAA TTT CAC ACC GC-3′) have been reported in the Japanese population. However, since the IVS6+35G > A and IVS1+11C > T had been found to be in complete linkage disequilibrium with IVS14–40G > C and 1389G > C (Thr463Thr), respectively. The remaining six SNPs were initially selected as candidates. For the haplotype association analysis, a pilot study was carried out by genotyping the six SNPs in 30 healthy Japanese individuals. Analysis with the SNPAlyze Version 5.0 software revealed the five haplotypes with a frequency of more than 1%, and they comprised all of the total predicted haplotype variation. As we were able to distinguish these five haplotypes with four SNPs, namely, IVS1+11C > T, IVS6+35G > A, IVS10–2A > G, and 972G > C (Gln324His), the 4 SNPs were ultimately chosen as the haplotype-tagging SNPs.

Association between the IVS1+11C > T SNP and increased risk of CRC. The 685 cases and 778 controls were genotyped for the *MUTYH* SNPs by PCR-CTPP, and the accuracy of the genotyping was verified by sequencing five specimens for each genotype of each SNP. The concordance rate was 100% (data not shown). The frequencies of each SNP are summarized in Table 1. The genotypic distributions of all the SNPs detected were in HWE. The IVS1+11C > T SNP, whose functional role has never been investigated, was shown to be statistically significantly associated with increased CRC risk. The crude OR was 1.43 (95% CI, 1.012–2.030; $P = 0.042$). After adjustments for gender, age and place of residence, the OR was estimated to be 1.46 (95% CI, 1.024–2.069; $P = 0.036$) (Table 1). The *P*-value remained less than 0.05 after FDR adjustment (Table 1). No statistically significant differences in the frequency of any of the other three SNPs, IVS6+35G > A, IVS10–2A > G, and 972G > C, were observed between the cases and controls (Table 1). Furthermore, the association between the SNPs of *MUTYH* and the risk of CRC was examined by the anatomic subsite of the CRC. It showed that the IVS1+11 A/T + T/T genotypes were nearly statistically significantly associated with an increased risk of distal colon cancer risk (OR, 1.58; 95% CI, 0.984–2.544; $P = 0.058$) (Table 2). Since monoallelic mutation of G382D has recently been shown to be associated with CRC risk in Caucasians,[16] the 685 cases and 778 control subjects were also examined for G382D, but no homozygotes or heterozygotes for this mutation were detected (data not shown). Because the complete linkage disequilibrium between IVS1+11C > T and 1389G > C had already been reported,[19] the results suggested that the IVS1+11C > T and 1389G > C variants of *MUTYH* may confer susceptibility to CRC in the Japanese population.

Table 1. Genotypes of the four *MUTYH* single nucleotide polymorphisms (SNPs) and risk of colorectal cancer

Variation[†]	Genotype	No. of controls (%)/cases (%)	Not adjusted		Adjusted[‡]		
			OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	FDR adjusted *P*-value[§]
IVS1+11C > T	C/C	714 (91.8)/607 (88.6)	1.00 (reference)	–	1.00 (reference)	–	–
	C/T + T/T	64 (8.2)/78 (11.4)	1.43 (1.012–2.030)	0.042	1.46 (1.024–2.069)	0.036	0.036
IVS6+35G > A	G/G	628 (80.7)/539 (78.7)	1.00 (reference)	–	1.00 (reference)	–	–
	G/A	143 (18.4)/140 (20.4)	1.14 (0.880–1.480)	0.321	1.14 (0.878–1.485)	0.321	0.963
	A/A	7 (0.9)/6 (0.9)	1.00 (0.334–2.990)	0.998	0.97 (0.320–2.926)	0.953	>1.0
IVS10–2A > G	A/A	741 (95.2)/662 (96.6)	1.00 (reference)	–	1.00 (reference)	–	–
	A/G + G/G	37 (4.8)/23 (3.4)	0.70 (0.409–1.183)	0.178	0.67 (0.390–1.139)	0.138	0.276
972G > C (Gln324His)	G/G	215 (27.6)/194 (28.3)	1.00 (reference)	–	1.00 (reference)	–	–
	G/C	395 (50.8)/350 (51.1)	0.98 (0.771–1.250)	0.883	0.96 (0.751–1.223)	0.733	>1.0
	C/C	168 (21.6)/141 (20.6)	0.93 (0.692–1.251)	0.632	0.90 (0.670–1.220)	0.511	>1.0

[†]Nucleotide +1 is the A of the ATG-translation initiation codon. [‡]Adjustment was made for gender, 5-year age class, and residential area. [§]False discovery rate (FDR) adjusted *P*-value. CI, confidence interval; OR, odds ratio.

Table 2. *MUTYH* genotypes and the risk of colorectal cancer (CRC) stratified by anatomic subsite

Variation†	Genotype	Proximal colon‡ ($n = 150$)			Distal colon‡ ($n = 232$)			Rectum‡ ($n = 290$)		
		No.	OR (95% CI)	*P*-value	No.	OR (95% CI)	*P*-value	No.	OR (95% CI)	*P*-value
IVS1+11C > T	C/C	133	1.00 (reference)	–	203	1.00 (reference)	–	259	1.00 (reference)	–
	C/T + T/T	17	1.50 (0.843–2.664)	0.169	29	1.58 (0.984–2.544)	0.058	31	1.36 (0.861–2.154)	0.187
IVS6+35G > A	G/G	122	1.00 (reference)	–	178	1.00 (reference)	–	229	1.00 (reference)	–
	G/A	28	1.03 (0.655–1.629)	0.887	51	1.27 (0.884–1.835)	0.195	58	1.07 (0.757–1.510)	0.703
	A/A	0	–	0.984	3	1.52 (0.383–6.056)	0.551	3	1.15 (0.286–4.581)	0.848
IVS10–2A > G	A/A	143	1.00 (reference)	–	226	1.00 (reference)	–	281	1.00 (reference)	–
	A/G + G/G	7	0.98 (0.422–2.268)	0.959	6	0.48 (0.197–1.156)	0.101	9	0.62 (0.295–1.319)	0.216
972G > C (Gln324His)	G/G	34	1.00 (reference)	–	72	1.00 (reference)	–	83	1.00 (reference)	–
	G/C	95	1.57 (1.020–2.426)	0.040	105	0.78 (0.553–1.108)	0.167	144	0.90 (0.655–1.247)	0.538
	C/C	21	0.78 (0.434–1.405)	0.409	55	0.99 (0.654–1.484)	0.943	63	0.94 (0.639–1.392)	0.768

†Nucleotide +1 is the A of the ATG-translation initiation codon. ‡Adjustment was made for gender, 5-year age class, and residential area. CI, confidence interval; OR, odds ratio.

Table 3. Haplotype frequency based on the four *MUTYH* single nucleotide polymorphisms (SNPs) and risk of colorectal cancer

Haplotype†				Frequency (%)‡		Not adjusted		Adjusted§		
IVS1+11C > T	IVS6+35G > A	IVS10–2A > G	972G > C	Control	Case	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	FDR adjusted *P*-value¶
C	G	A	C	42.9	40.4	1.00 (reference)	–	1.00 (reference)	–	–
C	G	A	G	40.9	41.5	0.93 (0.791–1.089)	0.360	1.10 (0.936–1.293)	0.248	0.992
C	A	A	G	9.8	10.7	1.08 (0.840–1.397)	0.537	1.18 (0.910–1.519)	0.215	0.645
T	G	A	C	4.0	5.8	1.43 (1.005–2.029)	0.046	1.56 (1.098–2.228)	0.013	0.013
C	G	G	G	2.5	1.6	0.63 (0.370–1.079)	0.090	0.66 (0.387–1.136)	0.135	0.270

†Nucleotide +1 is the A of the ATG-translation initiation codon. ‡Inferred common haplotypes with frequency >1% are listed. §Adjustment was made for gender, 5-year age class, and residential area. ¶False discovery rate (FDR) adjusted *P*-values. CI, confidence interval; OR, odds ratio.

Table 4. *MUTYH* haplotypes and the risk of colorectal cancer (CRC) stratified by anatomic subsite

Haplotype†				Proximal colon‡		Distal colon‡		Rectum‡	
IVS1+11C > T	IVS6+35G > A	IVS10–2A > G	972G > C	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
C	G	A	C	1.00 (reference)	–	1.00 (reference)	–	1.00 (reference)	–
C	G	A	G	1.13 (0.855–1.484)	0.397	1.10 (0.871–1.389)	0.424	1.06 (0.856–1.311)	0.595
C	A	A	G	1.00 (0.634–1.587)	0.990	1.27 (0.885–1.816)	0.196	1.15 (0.822–1.594)	0.424
T	G	A	C	1.61 (0.904–2.879)	0.106	1.81 (1.131–2.884)	0.013	1.36 (0.854–2.166)	0.195
C	G	G	G	0.99 (0.430–2.296)	0.988	0.43 (0.166–1.116)	0.083	0.62 (0.296–1.316)	0.216

†Nucleotide +1 is the A of the ATG-translation initiation codon. ‡Adjustment was made for gender, 5-year age class, and residential area. CI, confidence interval; OR, odds ratio.

Association between the TGAC haplotype containing the IVS1+11T and increased risk of CRC. Haplotype-based association studies are known to have greater power than individual SNP-based association studies.[30] Haplotype analyses were carried out based on the genotyping data of the four SNPs of *MUTYH*, namely, IVS1+11C > T, IVS6+35G > A, IVS10–2A > G, and 972G > C. There were five haplotypes with a frequency greater than 1%, CGAC, CGAG, CAAG, TGAC, and CGGG (Table 3), and since the CGAC haplotype was detected in 42.7% of the controls (Table 3), the highest percentage, the CGAC haplotype was used as the reference haplotype, and the following statistical analysis was carried out using the SAS system. Consistent with the results for each SNP, the TGAC haplotype containing the IVS1+11T allele was statistically significantly associated with increased CRC risk. The crude OR was 1.43 (95% CI, 1.005–2.029; $P = 0.046$) and after adjustment for gender, age and place of residence, the OR was 1.56 (95% CI, 1.098–2.228; $P = 0.013$) (Table 3). The *P*-value remained less than 0.05 after FDR adjustment (Table 3). The results of subsite-specific analysis revealed a significant association between the TGAC haplotype and increased risk of distal colon cancer (OR, 1.81; 95% CI, 1.131–2.884; $P = 0.013$) (Table 4). These results suggested that the TGAC haplotype containing the c.36+11T SNP confers susceptibility to CRC, especially to distal colon cancer, in the Japanese population.

RT-PCR analysis and detection of a novel SNP –280G > A linked with IVS1+11C > T. The association between the IVS1+11C > T SNP of *MUTYH* and CRC risk suggested a functional difference between IVS1+11C and IVS1+11T. The IVS1+11C > T SNP is located in the boundary region between *MUTYH* exon 1 and intron 1, and many reports have suggested that gene variants in the neighborhood of the junction are often accompanied by abnormal splicing.[31–33] In order to investigate whether the IVS1+11C > T SNP affects the splicing of *MUTYH*, an RT-PCR analysis was carried out by using cDNAs from carriers of the IVS1+11C/T and C/C genotype. However, no splicing abnormalities were detected in the cases carrying the IVS1+11C/T genotype (Fig. 2). On the other hand, during our checking of the sequences around the first exon of *MUTYH* to exclude variation at the splice site, a novel SNP of –280G > A was detected in the sample carrying the IVS1+11C > T SNP (Fig. 1b,c). Further genotyping was carried out in all subjects,

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and –280G > A was found to be 100% linked with IVS1+11C > T. The –280G > A SNP was demonstrated to be in complete linkage disequilibrium with the IVS1+11C > T SNP ($r^2 = 1$) by the SNPAlyze Version 5.0 software. Since the –280G > A SNP and 1389G > C (Thr463Thr) SNP were both in complete linkage disequilibrium with the IVS1+11C > T SNP, the results suggested that the Japanese individuals with the –280 A/IVS1+11T/1389C alleles or the –280A – IVS1+11T – IVS6+35G – IVS10-2A – 972C – 1389C haplotype were significantly associated with increased CRC risk.

Discussion

In this Japanese population-based case-control study four *MUTYH* SNPs, namely, IVS1+11C > T, IVS6+35G > A, IVS10–2A > G, and 972G > C, were genotyped in 685 CRC cases and 778 controls. The frequency distribution of IVS1+11T and the IVS1+11T – IVS6+35G – IVS10-2A – 972C (TGAC) haplotype were significantly associated with increased CRC risk. Subsite-specific analysis showed that the IVS1+11C > T SNP was nearly statistically significantly associated ($P = 0.058$) and the TGAC haplotype were statistically significantly associated ($P = 0.013$) with an increased risk of distal colon, but not proximal colon or rectal cancer. Next, we found that the IVS1+11C > T SNP was in complete linkage disequilibrium with –280G > A. No aberrant splicing induced by IVS1+11T allele was detected by RT-PCR. Together with the previously detected 1389G > C (Thr463Thr), a SNP in complete linkage disequilibrium with IVS1+11C > T, our results suggested that individuals who have the *MUTYH* – 280 A/IVS1+11T/1389C alleles or the TGAC haplotype are more susceptible to CRC in the Japanese population.

The present study is the first Japanese population-based CRC case-control study to evaluate the association between the SNPs of *MUTYH* and the risk of CRC by the anatomic subsite of the colorectal cancer. The results demonstrate that the IVS1+11C > T SNP and TGAC haplotype confer susceptibility to distal colon cancer in the Japanese population. Since this study investigated the association between four SNPs and five haplotypes of *MUTYH* and the risk of CRC on the same set of samples, a method for multiple testing is applicable to this study. Therefore, the method of FDR was used for all of the results, and the statistical significance of the associations was found to remain essentially unchanged (Tables 1 and 3). These results coincide with the fact that tumors arising from different subsites of the colorectum differ in their population distribution, clinical features as well as genetic pathways.[34–37] It was suggested from our results that the IVS1+11 > T SNPs and TGAC haplotype of *MUTYH* may be involved in distal colon carcinogenesis and that the risk of cancer arising from each anatomic subsite of the colorectum may be modified by different genetic pathways. Further studies need to be conducted to elucidate the underlying mechanisms.

The development of CRC is a multistep, multifactor process.[38] Some studies have demonstrated that environmental factors and physical conditions may modify the genetic risk of CRC associated with SNPs.[39,40] This may also hold true for the CRC risk associated with *MUTYH* SNPs. In the present study, adjustment was made for gender, age and place of residence to evaluate the association between the *MUTYH* SNPs and CRC risk. The adjusted OR and 95% CI remained essentially unchanged after the adjustments as compared with the values obtained without the adjustments (Tables 1 and 3). Furthermore, when the body mass index, disease history, physical activity, dietary factors, smoking and alcohol consumption status were taken into consideration, the *P*-value for IVS1+11C > T was 0.058, a nearly significant value, and the *P*-value for the TGAC haplotype remained less than 0.05 (data not shown). This result suggested that the association between the *MUTYH* SNPs and the risk of CRC was not significantly modified by environmental factors or the physical condition.

In the present study, the 972G/C genotype was statistically significantly associated with increased risk of proximal colon, but not distal colon or rectal cancer (Table 2). The functional analysis revealed no difference between the C/C type and G/G type,[4] and the 972C allele is more frequently detected in Japanese and Chinese than in European populations as shown in the dbSNPs of the NCBI Entrez system. Taking this into consideration with our result, it could be suggested that the 972C allele may be inversely associated with the development of at least proximal colon cancer in the Japanese population. Alternatively, this inverse association of the 972C allele with the risk of proximal colon cancer in the Japanese population may arise from its interaction with other allele(s). The IVS10–2A > G SNP had been demonstrated to generate a protein without nuclear expression and the IVS10–2G allele was suggested to be associated with a low BER function in the cell nuclei and thereby, act as a risk allele for cancer. However, the results of analyses in this study revealed an OR of less than 0.7 (except for cancer of the proximal colon) for the IVS10–2G allele and the CGGG haplotype, which contains the IVS10–2G allele, although the *P*-value did not reach statistical significance (Tables 1–4). These results remained essentially unchanged even after adjustments for environmental factors and physical conditions (as described above). Investigation of some other additional clinical factors, such as pathological stage, recurrence or survival, might yield some association. On the other hand, some studies have suggested that SNPs of repair genes may be associated with reduced cancer risk or fewer recurrences, and that effective host DNA repair capacity may be associated with poorer survival.[41–43] These observations suggest that mutations in the repair genes may also be inversely associated with malignant alterations, in addition to their more widely recognized association with increased cancer risk. The inverse association of the IVS10–2A > G SNP detected in this study with colorectal cancer risk might be explained by the contention that individuals with the A allele may be more resistant to ROS or other stresses than individuals with the G allele, and that the A allele has a protective effect on cells with mutations, similar to the situation suggested by Wang *et al.* for the XRCC1 Arg194Trp variant, a SNP associated with a reduced risk for various types of cancers.[44] Research on the effect of *MUTYHβ* isoforms on the cellular responses to various mutagens are expected help in clarifying this issue.

Besides the RT-PCR experiment, reporter assay was also carried out to investigate whether the –280G > A and IVS1+11C > T SNPs may affect the promoter activity of *MUTYH*. The dual-luciferase reporter assay experiments detected high transcriptional activity of the region (–411~+356) of *MUTYH* (data not shown). This information will be of use for future analyses. The reporter plasmids containing the wild-type and mutant sequence for the responses to oxidative stress were also investigated using the colon cancer cell line HCT116. ROS was induced by glucose oxidase, menadione or H_2O_2 at appropriate concentrations and treatment durations. However, the two linked SNPs, –280G > A and IVS1+11C > T, did not affect the promoter activity in our setting (data not shown). This study did not detect any functional differences in the –280G > A/IVS1+11C > T/1389G > C SNPs, and there remains the possibility that the three SNPs might be linked with other SNPs and these SNPs might affect the susceptibility to CRC.

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Alcohol dehydrogenase and *aldehyde dehydrogenase* polymorphisms and colorectal cancer: The Fukuoka Colorectal Cancer Study

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Alcohol dehydrogenase and aldehyde dehydrogenase are key enzymes in alcohol metabolism and therefore may be of importance to colorectal cancer development. The present case–control study was conducted to determine the influence of *ADH2*, *ADH3* and *ALDH2* polymorphisms in Fukuoka, Japan, with 685 incident cases of histologically confirmed colorectal adenocarcinomas and 778 community controls selected randomly from the study area. Alcohol use was ascertained by in-person interview. Statistical adjustment was made for sex, age class, area, and alcohol use. Individuals with the allele *47Arg* of the *ADH2* polymorphism (slow metabolizers) had a statistically significant increase in risk, with an adjusted OR of 1.32 (95% CI = 1.07–1.63), compared with those having the *ADH2*47His/His* genotype. This association was not affected by the level of alcohol consumption. The *ADH3* polymorphism showed no measurable association with the risk of colorectal cancer on either overall analysis or stratified analysis with alcohol use. The heterozygous *ALDH2*487Glu/Lys* genotype was not associated with an increase in the risk of colorectal cancer (adjusted OR 0.89, 95% CI = 0.71–1.13) compared with the *ALDH2*487Glu/Glu* genotype. Rather unexpectedly, the homozygous *ALDH2*487Lys/Lys* genotype was related to a statistically significantly decreased risk of colorectal cancer (adjusted OR 0.55, 95% CI = 0.33–0.93). It is unlikely that acetaldehyde metabolism determined by *ALDH2* polymorphism contributes to the risk of colorectal cancer, whereas the role of *ADH2* polymorphism deserves further investigation. (*Cancer Sci* 2007; 98: 1248–1253)

Alcohol consumption has fairly consistently been related to an increased risk of colorectal cancer.[1] In a pooled analysis of eight cohort studies in North America and Europe, a consumption of ≥45 g of alcohol per day was associated with a 1.4-fold increase in the risk of colorectal cancer.[2] A positive association between alcohol and colon or colorectal cancer has also been observed in Asian countries,[3–7] with few exceptions.[8] However, uncertainty remains as to the biological mechanisms for the association between alcohol use and colorectal cancer.

Ethanol is first oxidized to acetaldehyde by ADH, and acetaldehyde is further metabolized to acetate by ALDH. Human ADH exhibits several isoenzymes, and functional polymorphisms are known for the *ADH2* and *ADH3* genes.[9] A polymorphism in exon 3 of the *ADH2* gene, resulting in an arginine to histidine substitution in codon 47, affects the enzyme activity substantially. Individuals that are homozygous for the *ADH2*47His* allele (previously called *ADH2*2*) metabolize ethanol 40 times faster than those homozygous for the *ADH2*47Arg* allele (previously called *ADH2*1*).[10] The enzyme activity of *ADH2*47His/Arg* genotype is in the intermediate range between the two homozygous genotypes.[11] The polymorphic site for the *ADH3* gene is *Ile349Val* in exon 8. Maximal velocity is 2.5-fold greater in individuals homozygous for the *ADH3*349Ile* allele (previously called *ADH3*1*) than in those homozygous for the *ADH3*349Val* allele (previously called *ADH3*2*).[10] The *ADH2*47His* allele is fairly common in Asian populations and rare in Caucasians, while the *ADH3*349Val* allele is more frequent in Caucasians than in Asians.[12] *ALDH2* is the gene encoding mitochondrial ALDH, which contributes the majority of acetaldehyde oxidation in human liver and contains a functional polymorphism of *Glu487Lys*, with the variant *ALDH2*487Lys* (previously called *ALDH2*2*) allele resulting in an inactive form. The *ALDH2*487Lys* allele is mainly found in Asian populations.[12,13]

Several studies have investigated the relation of genetic polymorphisms of these alcohol-metabolizing enzymes to colorectal cancer and adenomas. As regards the *ADH2* polymorphism and colorectal cancer, a moderate increase in the risk of colorectal cancer was observed for each of the *Arg/His* and *Arg/Arg* genotypes compared with the *His/His* genotype in Japan,[14] but not in Spain.[15] The *ADH3* polymorphism was unrelated to colorectal cancer in two studies of Caucasians,[16,17] but one of these suggested an effect modification of alcohol consumption.[16] Two studies have examined the relation between *ADH3* polymorphism and colorectal adenomas in Caucasians, producing inconsistent results.[18,19] Although there was no difference in the distribution of *ADH3* genotypes between adenoma cases and controls in these studies, one showed a moderate increase in the risk of adenoma in men and women with the *ADH3*349Ile/Ile* genotype compared with those with the *ADH3*349Ile/Val* or *ADH3*349Val/Val* genotypes when alcohol consumption was high,[18] whereas the other reported an increased risk of adenoma associated with the *ADH3*349Val* allele for men with high alcohol consumption.[19] Studies regarding the *ALDH2* polymorphism and colorectal cancer or adenomas have all been done in Japan.[14,20–23] An approximately 3-fold increase in the risk of colorectal cancer has been observed for *ALDH2*487Glu/Lys* versus *ALDH2*487Glu/Glu* among alcoholics.[20] Another study

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Abbreviations: ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; CI, confidence interval; HDL, high-density lipoprotein; OR, odds ratio; PCR–RFLP, polymerase chain reaction–restriction fragment length polymorphism.

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suggested a greater increase in the risk of colon cancer, not of rectal cancer, associated with high alcohol consumption among individuals with the *ALDH2*487Glu/Lys* genotype.[21] A small case–control study showed a positive interaction between high alcohol consumption and the *ALDH2*487Glu/Lys* genotype, particularly on the risk of rectal cancer.[22] In contrast, the *ALDH2* polymorphism did not show any measurable association with either colorectal cancer or adenomas in recent studies.[14,23]

The present paper examines the relation of the *ADH2*, *ADH3* and *ALDH2* polymorphisms to colorectal cancer in a case–control study in Japan, focusing on effect modification of alcohol consumption and gene–gene interaction.

Materials and Methods

The Fukuoka Colorectal Cancer Study is a case–control study of incident cases and community controls, with Fukuoka City and three adjacent areas as the catchment area. Details have been reported previously.[24] Described below are methods relevant to the present analysis. The study protocol was approved by the ethical committees of Kyushu University and of all but two of the participating hospitals. There was no ethical committee at the two hospitals, and the survey was done at these hospitals with permission from the director of each hospital.

Subjects. Cases comprised a consecutive series of patients with histologically confirmed incident colorectal adenocarcinomas who were admitted to two university hospitals or six affiliated hospitals for surgical treatment during the period from October 2000 to December 2003. Other eligibility criteria included the following characteristics: age of 20–74 years at the time of diagnosis, residence in the study area, no prior history of partial or total removal of the colorectum, familial adenomatous polyposis or inflammatory bowel disease. Of the total of 1053 eligible cases, 840 cases (80%) participated in the interview, and 685 (65%) gave informed consent to genotyping.

Eligibility criteria for controls were the same as described for cases except for two items, that is, having no diagnosis of colorectal cancer and age of 20–74 years at the time of selection. A total of 1500 persons were selected as control candidates using two-stage random sampling from among residents living in 15 small areas. A total of 833 persons participated in the survey, and 778 gave informed consent to genotyping. Reasons for exclusion and non-participation were death ($n = 7$), migration from the study area ($n = 22$), undelivered mail ($n = 44$), mental incompetence ($n = 19$), history of partial or total removal of the colorectum ($n = 21$), diagnosis of colorectal cancer after the survey ($n = 5$), no response ($n = 158$), and refusal ($n = 391$). After exclusion of the first six categories of outcomes ($n = 118$), net participation rates were calculated as 60% (833/1382) for the interview and 56% (778/1382) for genotyping.

Interview. Research nurses interviewed cases and controls in person regarding physical activity, smoking, alcohol use, and other factors using a uniform questionnaire. Habitual alcohol consumption at the time 5 years prior to the onset of disease in cases or the interview in controls was ascertained. Individuals reported the average number of days per week that alcohol was consumed and the average amount of alcohol per day of drinking. 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 a unit. The reproducibility of the questionnaire was tested on 29 control subjects (14 men and 15 women) with an interval of approximately 1 year, and the reported alcohol intake was highly reproducible (Spearman's $r = 0.82$).

Genotyping. A venous blood sample of 5 mL was taken after the interview. DNA was extracted from the buffy coat using a commercial kit (QIAGEN GmbH, Hilden, Germany) and genotyping was performed using the PCR–RFLP method. The PCR was performed in a reaction mixture of 10 μL containing 0.5 IU of Taq and 1 μL of template DNA with a concentration of approximately 50–150 ng/μL. The *ADH2 Arg47His* and *ADH3 Ile349Val* genotypes were determined according to the methods described by Osier *et al.*[25] Primers for the *ADH2 Arg47His* genotypes were 5′-ATT CTA AAT TGT TTA ATT CAA GAA g-3′ (sense) and 5′-ACT AAC ACA GAA TTA CTG GAC-3′ (antisense). PCR products were digested with 20 IU of *MslI* for 16 h at 37°C in a mixture of 20 μL, resulting in fragments of 443 bp and 242 bp for the *47His* allele and 685 bp for the *47Arg* allele. The *ADH3 Ile349Val* genotypes were determined using primers of 5′-TTG TTT ATC TGT GAT TTT TTT TGT-3′ (sense) and 5′-CGT TAC TGT AGA ATA CAA AGC-3′ (antisense). The PCR product of 378 bp fragments was digested with 5 IU of *SspI* in a reaction mixture of 20 μL for 3 h at 37°C, resulting in fragments of 274 bp and 104 bp for the *349Ile* allele and 378 bp for the *349Val* allele. The *ALDH2 Glu487Lys* genotypes were determined, as described by Goedde *et al.*,[26] using primers that were 5′-CAA ATT ACA GGG TCA ACT GCT-3′ (sense) and 5′-CCA CAC TCA CAG TTT TCT CTT-3′ (antisense). The PCR product was digested with *Ksp632I* (10 IU) or *EarI* (10 IU) for 12 h at 37°C in a mixture of 20 μL, resulting in fragments of 112 bp for the *ALDH2*487Glu* allele and 135 bp for the *ALDH2*487Lys* allele. The digested PCR products were separated using electrophoresis on 3% agarose gels (NuiSieve GTG, Rockland, ME, USA), and visualized with ethidium bromide.

Statistical analysis. The association of the genetic polymorphisms with risk of colorectal cancer was examined using multiple logistic regression analysis including indicator variables for sex, 5-year age class (the lowest class was <40 years), resident area (Fukuoka City or adjacent areas), and alcohol intake (0, 0.1–1.9, or ≥2.0 units per day) as covariates. Adjusted OR and 95% CI were obtained from the logistic regression coefficient and the standard error for the corresponding indicator variable. Statistical significance for the interaction was tested using the likelihood ratio test comparing the logistic models with and without interaction terms for the genotype and alcohol category. Statistical significance was concluded if the two-sided *P*-value was less than 0.05 or if the 95% CI did not include unity. All statistical analyses were performed using SAS version 8.2 (SAS Institute Inc., Cary, NC, USA).

Results

The number of men among the 685 cases and 778 controls was 426 (62%) and 490 (63%), respectively. The mean age of the cases was 60 years (range 27–74), and that of the controls was 59 years (range 22–75). More than half of the cases (61%) and controls (64%) were residents of Fukuoka City. All of the distributions of genotypes for the *ADH2 Arg47His*, *ADH3 Ile349Val*, and *ALDH2 Glu487Lys* polymorphisms were in agreement with the Hardy–Weinberg equilibrium in both cases and controls. The alcohol-drinking pattern differed strikingly by *ALDH2* polymorphism and slightly so with respect to the *ADH2* polymorphism (Fig. 1). Alcohol use was progressively less frequent with increasing numbers of the *ALDH2*487Lys* allele, and was slightly more frequent with increasing numbers of the *ADH2*47Arg* allele. There was no variation in the proportion of alcohol drinking according to the *ADH3 Ile349Val* polymorphism (data not shown).

Regarding the *ADH2* polymorphism, the *47Arg* allele was slightly more frequent in cases than in controls, and the adjusted OR for the *Arg/His* and *Arg/Arg* genotypes as compared with the *His/His* genotype were each greater than unity, the increase for the heterozygote being statistically significant (Table 1). The adjusted OR for those with the *ADH2*47Arg* allele compared

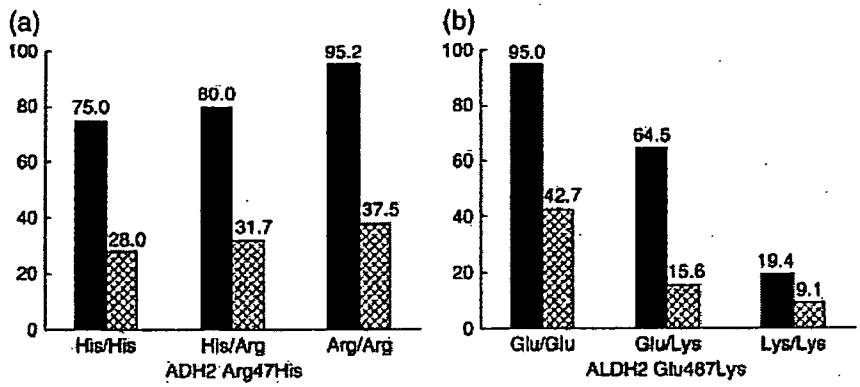

Fig. 1. Proportions (%) of alcohol drinkers for men (gray bar) and women (hatched bar) according to the *ADH2 Arg47His* and *ALDH2 Glu487Lys* polymorphisms in the control group. Values shown at the top of each bar are percentages of alcohol drinkers. Trend *P*-values were 0.03 in men and 0.35 in women for the *ADH2* polymorphism, and <0.0001 in both sexes for the *ALDH2* polymorphism. The trend *P*-value was based to the Mantel-Haenszel method with scores of 0, 1, and 2 assigned for the number of the variant allele.

Table 1. Relation of *ADH2*, *ADH3*, and *ALDH2* polymorphisms to colorectal cancer risk

Genotype	Cases (*n*, %)	Controls (*n*, %)	Adjusted OR (95% CI)†
ADH2 Arg47His‡			
His/His (fast)	345 (50.8)	452 (58.1)	1.00 (referent)
Arg/His	294 (43.3)	289 (37.1)	1.32 (1.06–1.64)
Arg/Arg (slow)	40 (5.9)	37 (4.8)	1.36 (0.84–2.20)
ADH3 Ile349Val§			
Ile/Ile (fast)	609 (88.9)	706 (90.9)	1.00 (referent)
Ile/Val	74 (10.8)	68 (8.7)	1.29 (0.91–1.83)
Val/Val (slow)	2 (0.3)	3 (0.4)	0.77 (0.13–4.70)
ALDH2 Glu487Lys			
Glu/Glu	400 (58.4)	416 (53.5)	1.00 (referent)
Glu/Lys	257 (37.5)	309 (39.7)	0.89 (0.71–1.13)
Lys/Lys (null activity)	28 (4.1)	53 (6.8)	0.55 (0.33–0.93)

†Adjusted for sex, 5-year age class, area, and alcohol use. ‡Six cases were excluded because of undetermined genotype. §One control was excluded because of undetermined genotype. CI, confidence interval; OR, odds ratio.

with those without was 1.32 (95% CI = 1.07–1.63). There was no measurable difference in the distribution of *ADH3 Ile349Val* genotypes between cases and controls. The *ALDH2*487Lys* allele was less frequent in cases than in controls, and the adjusted OR of colorectal cancer for the *Lys/Lys versus Glu/Glu* genotype was statistically significantly lower than unity. Analysis by sex showed similar results for men and women. For instance, the adjusted OR for *ADH2*47Arg/His* and *Arg/Arg* genotypes combined were 1.34 (95% CI = 1.03–1.75) in men and 1.35 (95% CI = 0.95–1.91) in women. Regarding the *ALDH2* polymorphism, the adjusted OR for the *Glu/Lys* and *Lys/Lys* genotypes compared with the *Glu/Glu* genotype in men were 0.77 (95% CI = 0.56–1.05) and 0.44 (95% CI = 0.22–0.91), respectively, while the corresponding values in women were 1.04 (95% CI = 0.71–1.53) and 0.67 (95% CI = 0.31–1.46), respectively.

Table 2 summarizes the results from the analysis regarding the interaction between alcohol intake and each genetic polymorphism. In this analysis, individuals heterozygous for the *ADH2* or *ADH3* polymorphism were each combined with those homozygous for the variant allele. Individuals homozygous for the variant allele of *ALDH2* (*487Lys/Lys*) were excluded because alcohol use was almost null in this group. There was no appreciable effect modification of each polymorphism on the relation between alcohol and colorectal cancer. High alcohol consumption was related to a moderate increase in the OR of colorectal cancer regardless of the genotype. Adjusted OR for high alcohol use (≥2 units/day) versus no use after control for the *ADH2*, *ADH3*, and *ALDH2* genotypes each were 1.34 (95% CI = 0.99–1.82), 1.37 (95% CI = 1.01–1.85) and 1.20 (95% CI = 0.85–1.69), respectively.

The joint effects of the *ADH2* or *ADH3* polymorphism in combination with the *ALDH2* polymorphism were examined among alcohol drinkers (Table 3). Individuals with the *ADH2*47His/His* genotype and the *ALDH2*487Lys* allele showed a statistically non-significant, small decrease in the OR for colorectal cancer. No such decrease was noted on analysis of the non-alcohol drinkers (data not shown). There was no clear interaction between the *ADH3* and *ALDH2* polymorphisms on the risk of colorectal cancer in either non-alcohol drinkers or alcohol drinkers.

Discussion

The present study addressed the relation between genetic polymorphisms in alcohol metabolism and colorectal cancer. Individuals with the *ADH2*47Arg* allele (slow metabolizers) showed a modest, but statistically significant, increase in the risk of colorectal cancer. In contrast, individuals homozygous for the *ALDH2* variant allele had a decreased risk of colorectal cancer. None of the polymorphisms affected the relation between alcohol and colorectal cancer. Because of the limited variation in the *ADH3* polymorphism, the present study did not provide useful information regarding the role of *ADH3* polymorphism in colorectal carcinogenesis.

Both *ADH2* and *ADH3* polymorphisms have been shown to affect the risk of various alcohol-related conditions. The slow alcohol metabolism of *ADH3* polymorphism has been related to increased risk of alcoholism and liver cirrhosis, elevated levels of serum HDL cholesterol, and decreased risk of myocardial infarction in Western populations.[27,28] Studies in Japan have reported that the slow metabolizers with the *ADH2* polymorphism had an increased risk of alcoholic liver disease,[29] and of cerebral infarction.[30] However, it remains uncertain whether these polymorphisms affect the risk of alcohol-related cancers. In a meta-analysis of seven case–control studies, no association between *ADH3*

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Table 2. Combined effects of *ADH2*, *ADH3*, and *ALDH2* polymorphisms with alcohol use on the risk of colorectal cancer

Genotype		Alcohol intake (unit/day)†		
		0	<2	≥2
ADH2 Arg47His				
His/His	No.‡	142/192	109/167	94/93
	OR (95% CI)§	1.00 (referent)	0.97 (0.69–1.37)	1.52 (1.02–2.25)
Arg/His + Arg/Arg	No.	128/119	109/123	97/84
	OR (95% CI)	1.46 (1.04–2.03)	1.30 (0.92–1.85)	1.69 (1.14–2.52)
		Interaction $P = 0.61$		
ADH3 Ile349Val				
Ile/Ile	No.	235/281	199/266	175/159
	OR (95% CI)	1.00 (referent)	0.98 (0.74–1.28)	1.44 (1.05–1.98)
Ile/Val + Val/Val	No.	37/30	22/23	17/18
	OR (95% CI)	1.49 (0.89–2.50)	1.29 (0.70–2.40)	1.27 (0.62–2.57)
		Interaction $P = 0.49$		
ALDH2 Glu487Lys¶				
Glu/Glu	No.	98/103	150/171	152/142
	OR (95% CI)	1.00 (referent)	1.04 (0.71–1.53)	1.24 (0.81–1.90)
Glu/Lys	No.	147/163	71/112	39/34
	OR (95% CI)	1.02 (0.71–1.48)	0.74 (0.47–1.17)	1.33 (0.74–2.39)
		Interaction $P = 0.30$		

†One unit of alcohol intake corresponded to 1 go (180 mL) of *sake*, 0.5 go (90 mL) of *shochu*, 1 large bottle (633 mL) of beer, 2 drinks (60 mL) of whiskey, or 2 glasses (200 mL) of wine. ‡Numbers of cases/controls. §Adjusted for sex, 5-year age class, and area. ¶Individuals with 487Lys/Lys genotype were excluded, because alcohol drinkers were few. CI, confidence interval; OR, odds ratio.

Table 3. Combined effects of *ADH2* or *ADH3* polymorphism with the *ALDH2* polymorphism on the risk of colorectal cancer in alcohol drinkers

ADH2/ADH3		*ALDH2 Glu487Lys*	
		Glu/Glu	*Glu/Lys*
ADH2 Arg47His			
His/His	No.†	153/170	50/84
	OR (95% CI)‡	1.00 (referent)	0.70 (0.46–1.07)
Arg/His + Arg/Arg	No.	148/143	57/62
	OR (95% CI)	1.12 (0.81–1.55)	1.08 (0.70–1.67)
		Interaction $P = 0.30$	
ADH3 Ile349Val			
Ile/Ile	No.	274/283	100/135
	OR (95% CI)	1.00 (referent)	0.83 (0.60–1.14)
Ile/Val + Val/Val	No.	28/30	10/11
	OR (95% CI)	1.00 (0.58–1.74)	0.98 (0.40–2.38)
		Interaction $P = 0.76$	

†Numbers of cases/controls. ‡Adjusted for sex, 5-year age class, area, and alcohol use low or high intake. CI, confidence interval; OR, odds ratio.

polymorphism and upper aerodigestive cancers was found, nor was any interaction between *ADH3* polymorphism and alcohol consumption on the risk of these cancers.[12] A study of Japanese alcoholics has showed increased risks of oral, laryngeal, and esophageal cancer for the *ADH2*47Arg/Arg* genotype,[31] while another study in Japan showed no effect modification of the *ADH2* polymorphism on the association between alcohol and esophageal cancer.[32]

A recent Japanese study reported a positive association between the *ADH2* polymorphism and colorectal cancer, showing a progressive increase in the risk with increasing numbers of the *ADH2*47Arg* allele.[14] No such progressive increase in the risk was observed in the present study, but the authors' findings are compatible with the previous observation in that the risk was elevated in individuals with the *ADH2*47Arg* allele. The authors have no clear explanation for the increased risk of colorectal cancer associated with the *ADH2* polymorphism, but the consistency in the two independent studies warrants further investigation regarding the role of the *ADH2* polymorphism in colorectal carcinogenesis.

The present study showed neither an increased risk of colorectal cancer associated with the *ALDH2*487Lys* allele nor interaction between the *ALDH2*487Lys* allele and alcohol consumption. These findings are at odds with results from previous studies of colorectal cancer,[20–22] but are consistent with the recent observations on colorectal cancer,[14] and adenomas.[23] The statistically significant decrease in the risk of colorectal cancer associated with the *ALDH2*487Lys/Lys* genotype was rather unexpected and difficult to interpret. A decrease in the OR associated with the *Lys/Lys* genotype was also observed in the analysis confined to non-drinkers of alcohol ($n = 583$), although the decrease was not statistically significant; adjusted OR for the *Glu/Glu*, *Glu/Lys*, and *Lys/Lys* genotypes were 1.00 (referent), 1.00 (95% CI = 0.68–1.46), and 0.64 (95% CI = 0.36–1.14), respectively. The decreased risk in individuals with the *ALDH2487Lys/Lys* genotype may have been due to residual confounding of lifestyle factors other than alcohol drinking. In the Fukuoka Colorectal Cancer Study, obesity and physical inactivity were related to increased risk,[33] and there was a protective association with intake of n-3 polyunsaturated fatty acids.[34] With further

adjustment for these factors as well as for dietary calcium and fiber using the variables and categories as defined previously,[34] the adjusted OR for the *Glu/Lys* and *Lys/Lys* genotypes versus the *Glu/Glu* genotype were 0.90 (95% CI = 0.70–1.14) and 0.52 (95% CI = 0.31–0.88), respectively, in the analysis excluding four cases and two controls with a total calorie intake estimated to be >20 929 kJ/day. While a similar, inverse association was noted for colorectal adenomas,[23] no such association was seen in another study of colorectal cancer in Japan.[14]

It is hypothesized that acetaldehyde is accumulated in individuals who are fast alcohol metabolizers and slow acetaldehyde metabolizers.[29] Thus the authors hypothesized that the combination of *ADH2*His/His* and *ALDH2*487Glu/Lys* genotype might be related to an increased risk of colorectal cancer, but the risk of colorectal cancer was decreased, rather than increased in alcohol drinkers with such composite genotypes.

This finding on the gene–gene interaction, together with the above-mentioned findings on the *ALDH2* polymorphism, suggests that acetaldehyde metabolism in the liver is not measurably linked to colorectal carcinogenesis. Bacterial production of acetaldehyde in the colon is an alternative mechanism by which alcohol may enhance colorectal carcinogenesis. Human colonic contents and isolated colonic microbes are capable of producing acetaldehyde when incubated with ethanol *in vitro*.[35,36] It has been demonstrated in piglets that high levels of acetaldehyde were produced in the colon during normal metabolism of alcohol.[37] ALDH activity is much lower in the colonic mucosa than in the liver,[38,39] and colonic epithelial cells are exposed to high concentrations of acetaldehyde in the colonic lumen. It is hypothesized that low folate status increases the risk of colorectal cancer by altering DNA methylation and DNA synthesis.[40,41] Alcohol and acetaldehyde exert adverse effects on folate metabolism.[42] High alcohol consumption results in inadequate folate status by decreasing intestinal absorption and increasing renal excretion. It is known that acetaldehyde rather than alcohol itself cleaves folate chemically. Ethanol ingestion has resulted in a substantial increase in the intracolonic concentration of acetaldehyde and decreased folate levels in the colonic mucosa in an experimental study of rats.[43]

The present study is probably the largest that has ever been reported regarding the *ADH2* or *ALDH2* polymorphism and colorectal cancer. Among the reported studies are those including 257 colorectal cancer cases and 771 controls,[14] 270 cases and 121 controls,[21] and 142 cases and 241 non-cancer controls,[22] in Japan. The size of a study is particularly important in investigating the role of rare genotypes in the gene–environment or gene–gene interaction. The participation rate in terms of genotyping was not so high in either cases (65%) or controls (56%). Because the *ADH2* and *ALDH2* polymorphisms affected alcohol drinking, a selection bias would be possible in the association with these polymorphisms if cases and controls participated in the study differentially with respect to alcohol drinking. Among those interviewed, however, the proportions of alcohol drinking in the cases and controls each did not differ by consent to genotyping.[44] Alcohol consumption 5 years prior to the referent date was used, and the authors have no data as to how valid the recalled alcohol consumption in the past was, although it was found to be highly reproducible.

In summary, a case–control study in Japan showed an increased risk of colorectal cancer associated with the *ADH2*47Arg* allele, but not with the *487Lys* allele of *ALDH2* polymorphism. None of the polymorphisms affected the relation between alcohol consumption and colorectal cancer risk. It is unlikely that acetaldehyde metabolism determined by *ALDH2* polymorphism contributes to the risk of colorectal cancer, whereas the role of *ADH2* polymorphism deserves further investigation.

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Colorectal Polypectomy and Risk of Colorectal Cancer by Subsite: The Fukuoka Colorectal Cancer Study

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Background: Colorectal adenomas are well-established precursor lesions for colorectal cancer and removal of polyps is deemed to reduce the risk of colorectal cancer. However, benefit of colorectal polypectomy in routine practice is still uncertain. We therefore investigated subsite-specific risks of colorectal cancer in relation to history of colorectal polypectomy in a case-control study.

Methods: Both case patients and control subjects were residents aged 20–74 years in Fukuoka City and three adjacent areas. The case group comprised 840 patients undergoing surgery for a first diagnosis of colorectal cancer, while the control subjects were 833 residents who were selected in the community by two-stage random sampling. Past history of selected diseases, surgery and lifestyle factors were ascertained by in-person interview. Statistical adjustment was made for sex, 5-year age class, residence, smoking, alcohol drinking, physical activity, body mass index and parental history of colorectal cancer.

Results: Overall, 74 case patients (9%) and 85 control subjects (10%) reported a prior history of colorectal polyps, and 50 cases (6%) and 64 controls (8%) had a history of colorectal polypectomy. The adjusted odds ratio associated with colorectal polypectomy was 0.71 (95% confidence interval [CI] 0.48–1.06) for the overall risk of colorectal cancer. The corresponding values for cancer of the proximal colon, distal colon, and rectum were 1.68 (95% CI 0.98–2.88), 0.71 (95% CI 0.41–1.26) and 0.24 (95% CI 0.11–0.52), respectively.

Conclusions: The findings indicate that colorectal polypectomy in current practice confers a decreased risk of rectal cancer and possibly of distal colon cancer.

Key words: colorectal cancer – colorectal polypectomy – case-control study – Japanese

INTRODUCTION

Colorectal cancer is one of the most common cancers in the world, accounting for nearly 10% of all incident cases of cancer (1). In Japan, mortality from colorectal cancer, especially from colon cancer, has increased markedly during

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the past 50 years (2), and Japan is currently among the countries with the highest incidence rates of colorectal cancer worldwide (3). Because colorectal adenomas are well-established precursor lesions for colorectal cancer, it is recommended to remove polyps when detected, search the colon for additional polyps, and arrange for long-term surveillance of the subjects (4).

Adenomas, particularly of large size and with any villous component and dysplasia, are often accompanied by cancerous lesions (5–7). Progression of polyps to cancer has been documented in an early follow-up study of patients with unresected lesions (8), although clearly many do not develop to cancer (9). The adenoma–carcinoma sequence is also supported by progressive accumulation of genetic alterations involved in colorectal carcinogenesis (10). Furthermore, patients undergoing colorectal polypectomy have been found to exhibit a lower incidence of colorectal cancer than the general population in different countries (11–13). It is, however, uncertain to what extent colorectal polypectomy confers reduction in the risk of colorectal cancer in Japan. Only a small prospective study suggested a lower risk of colorectal cancer among patients undergoing polypectomy as compared with those with their polyps unresected (14). In the present study we therefore examined the relation between a history of colorectal polypectomy and subsequent risk of colorectal cancer in the Fukuoka Colorectal Cancer Study (15).

SUBJECTS AND METHODS

The Fukuoka Colorectal Cancer Study is a case-control study of incident cases of colorectal cancer at eight large hospitals in the study area (Fukuoka City and three adjacent areas) and community controls. The study protocol was approved by the ethical committee of the Faculty of Medical Sciences, Kyushu University. Details of the methods have been described elsewhere (15). Methodological issues relevant to the present study are briefly described below.

Subjects

Cases comprised a consecutive series of patients with histologically confirmed incident cases of colorectal adenocarcinoma, who were admitted to two university hospitals or six affiliated hospitals for surgical treatment during the period from September 2000 to December 2003. Eligibility criteria included the following characteristics: age of 20–74 years at the time diagnosis, residence in the study area, no prior history of partial or total removal of the colorectum, familial adenomatous polyposis or inflammatory bowel disease, mental competence to give informed consent and to complete the interview. Research nurses visited each hospital weekly and determined eligibility of cases by referring to admission logs and medical records. Of 1053 eligible cases, 840 (80%) participated in the survey. Numbers of the interviewed cases according to the locations of 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 colon.

Eligibility criteria for controls were the same as described for cases except for the two items, i.e. having no diagnosis of colorectal cancer and age of 20–74 years at the time of selection. A total of 1500 persons were selected as control candidates by two-stage random sampling, by frequency matching to the expected distribution of incident cases with respect to sex and 10-year age class. The first step was a random selection of 15 small areas out of 178 in total and approximately 100 persons were randomly selected in each small area. At most four additional letters of invitation were mailed to control candidates. A total of 833 persons participated in the survey. Reasons for exclusion and nonparticipation were death ($n = 7$), migration from the study area ($n = 22$), undelivered mail ($n = 44$), mental incompetence ($n = 19$), history of partial or total removal of the colorectum ($n = 21$), diagnosis of colorectal cancer after the survey ($n = 5$), no response ($n = 158$), and refusal ($n = 391$). After exclusion of the first six categories of outcomes ($n = 118$), the net participation rate was calculated as 60% (833/1382). Interviews were conducted at community halls, collaborative clinics, or other places.

Interview

Research nurses interviewed cases and controls in person regarding physical activity, smoking, alcohol use, parental history of colorectal cancer, past history of selected diseases, and bowel habit by using a uniform questionnaire. Most of the questions were closed-ended, and some of the quantitative questions were open-ended. Prior histories of physician's diagnosis and surgery included colorectal polyp and colorectal polypectomy among others.

Statistical Analysis

The association of a history of colorectal polyp and polypectomy with the risk of colorectal cancer was examined by use of multiple logistic regression analysis. Statistical adjustment was made for gender, 5-year age class, resident area (Fukuoka City or suburban area), alcohol drinking, cigarette smoking, occupational physical activity, leisure-time physical activity, body mass index and parental history of colorectal cancer. Cigarette smoking was categorized into 0, 1–399, 400–799, and 800+ cigarette-years, which were calculated by multiplying the average number of cigarettes smoked per day by years of smoking. Alcohol use was categorized into 0, 0.1–0.9, 1.0–1.9 and 2.0+ units per day on the basis of the average amount of alcohol per day 5 years before; one unit corresponded roughly to one large bottle of beer (633 ml) or one go of sake (180 ml). Occupational physical activity was classified with sedentary and non-sedentary jobs. Leisure-time physical activity was evaluated in terms of a metabolic equivalent (MET)-hour score; the MET

(intensity of physical activity) of an individual type of physical activity was multiplied by hours spent for the activity. Body mass index was calculated by using body weight 10 years before and body mass index were categorized by using quartiles in the distribution in the controls. Adjusted odds ratio (OR) and 95% confidence interval (CI) were obtained from the logistic regression coefficient and its standard error for an indicator variable representing a specific category of the covariate. Statistical significance was declared if 95% CI did not include unity. All statistical analyses were done using the SAS version 8.2 (SAS Institute Inc., Cary, NC).

RESULTS

Males numbered 501 (60%) in the cases and 515 (62%) in the controls. Mean age of the cases was 61 years (range 27–74), and that of the controls was 59 years (range 22–75). Residents in Fukuoka City accounted for 61% of the cases and 65% of the controls. There were 74 cases and 86 controls who reported a history of physician's diagnosis of colorectal polyps, and 50 cases and 64 controls had a history of colorectal polypectomy (Table 1). The years of these two events (diagnosis and polypectomy) were identical with 49 cases and 59 controls each, and the diagnosis of polyp preceded 1–9 years prior to polypectomy in the remaining of cases and controls. The overall risk of colorectal cancer was moderately lowered in individuals with a history of colorectal polypectomy; adjusted odds ratios associated with colorectal polypectomy was 0.71 (95% CI 0.48–1.06).

Table 2 shows results from the analysis by location. Cases with multiple-site cancers were excluded. A statistically significant decrease in the risk associated with a history of colorectal polypectomy was observed for rectal cancer. A less marked, statistically non-significant decrease in the risk of distal colon cancer was also seen among those with an episode of colorectal polypectomy. The risk of proximal colon cancer was slightly increased among those with a history of colorectal polypectomy. Adjusted odds ratios associated with colorectal polypectomy for cancer of the proximal colon, distal colon and rectum were 1.68 (95% CI 0.98–2.88), 0.71 (95% CI 0.41–1.26) and 0.24 (95% CI 0.11–0.52), respectively.

Although the majority of the subjects reporting a history of colorectal polyp had a history of colorectal polypectomy, we stratified the subjects with a history of colorectal polyp by the presence of polypectomy (Table 3). Adjusted ORs for those having a history of colorectal polyp but not of colorectal polypectomy did not measurably differ from unity. However, the risks of colorectal cancer and subsite-specific cancers among those reporting colorectal polypectomy were almost the same as described above.

Medians of the duration after colorectal polypectomy among the 50 cases and 64 controls with a history of colorectal polypectomy were each 4.0 years (range 0–15). We examined the association of colorectal polypectomy with overall and subsite-specific risks of colorectal cancer according to the length in time (<5 and 5+ years) after colorectal polypectomy. In individuals with colorectal polypectomy within 5 years before, adjusted ORs of colorectal, proximal colon, distal colon and rectal cancers were 0.59 (95% CI 0.35–0.99), 1.13 (95% CI 0.53–2.38), 0.72 (95% CI 0.36–1.48) and 0.24 (95% CI 0.09–0.62), respectively. The corresponding values for those having undergone polypectomy 5 or more years before were 0.89 (95% CI 0.50–1.59), 2.53 (95% CI 1.22–5.22), 0.67 (95% CI 0.27–1.63) and 0.25 (95% CI 0.07–0.84), respectively.

Table 1. Risk of colorectal cancer in relation to a history of colorectal polyp development and polypectomy

Past history	No. (%) of cases	No. (%) of controls	Crude OR (95% CI)	Adjusted OR (95% CI)*
Colorectal polyp				
None	766 (91.2)	747 (89.8)	1.00 (referent)	1.00 (referent)
Present	74 (8.8)	86 (10.2)	0.84 (0.61–1.16)	0.77 (0.55–1.08)
Colorectal polypectomy				
None	790 (94.0)	769 (92.3)	1.00 (referent)	1.00 (referent)
Present	50 (6.0)	64 (7.7)	0.76 (0.52–1.12)	0.71 (0.48–1.06)

OR, odds ratio; CI, confidence interval.
*Adjusted for sex, 5-year age class, residence, cigarette smoking, alcohol drinking, occupational physical activity, leisure-time physical activity, body mass index, and parental history of colorectal cancer.

DISCUSSION

The present study showed that a history of colorectal polypectomy was associated with a substantial decrease in the risk of rectal cancer, and there was also a suggestive, protective association between colorectal polypectomy and distal colon cancer. The present findings indicate that colorectal polypectomy in the current practice is protective against cancer at the distal segment of the colorectum, but not in the proximal colon in Japan.

Because we did not ascertain details regarding the method of colorectal examination by which polyps were diagnosed and the location of polyps removed in the present study, it is difficult to explain why the decreased risk associated with colorectal polypectomy was only limited to rectal and distal colon cancers. Detection of the majority of colorectal polyps was probably on the basis of the fecal occult blood (FOB) screening and it could be speculated that the FOB screening may have resulted in a greater detection of polyps at the distal segment. However, it was shown that the FOB screening was as effective for cancers proximal to the sigmoid colon as for distal cancers (16,17). It was also observed that the FOB screening was associated with reduced mortality from colon and rectal cancers equally (18,19). Polyps of the

Table 2. Risks of proximal colon, distal colon, and rectal cancers in relation to a history of colorectal polyp development and polypectomy

	Proximal colon		Distal colon		Rectum	
Past history	No.	OR (95% CI)*	No.	OR (95% CI)*	No.	OR (95% CI)*
Colorectal polyp						
None	164	1.00 (referent)	256	1.00 (referent)	332	1.00 (referent)
Present	27	1.39 (0.85–2.27)	23	0.67 (0.40–1.10)	22	0.53 (0.32–0.87)
Colorectal polypectomy						
None	168	1.00 (referent)	261	1.00 (referent)	346	1.00 (referent)
Present	23	1.68 (0.98–2.88)	18	0.71 (0.41–1.26)	8	0.24 (0.11–0.52)

*OR, odds ratio; CI, confidence interval; Adjusted for sex, 5-year age class, residence, cigarette smoking, alcohol drinking, occupational physical activity, leisure-time physical activity, body mass index, and parental history of colorectal cancer.

rectum and distal colon may have been more effectively diagnosed and removed than proximal colon polyps in the present study population. Two case-control studies in the USA (20,21) investigated the association between screening sigmoidoscopy and the risk of colorectal cancer and reported that decreased risk associated with sigmoidoscopy was observed almost exclusively for cancer of the rectum and sigmoid colon. In another case-control study in the USA, however, decreased risks of both colon and rectal cancers were observed for any endoscopic procedures (22). Screening endoscopy with no distinction between sigmoidoscopy and colonoscopy was also associated with a marked decrease in the overall risk of colorectal cancer in a case-control study in Germany (23).

Table 3. Risks of colorectal cancer by subsite according to the combination of histories of colorectal polyp development and polypectomy

	Polyp (–)	Polyp (+)	
		Polypectomy (–)	Polypectomy (+)
All cases			
No.	766	24	50
OR (95% CI)*	1.00	0.94 (0.52–1.70)	0.71 (0.48–1.06)
Proximal colon cancer			
No.	164	4	23
OR (95% CI)*	1.00	0.72 (0.24–2.17)	1.67 (0.97–2.85)
Distal colon cancer			
No.	256	5	18
OR (95% CI)*	1.00	0.56 (0.21–1.52)	0.70 (0.40–1.24)
Rectal cancer			
No.	332	14	8
OR (95% CI)*	1.00	1.37 (0.68–2.76)	0.25 (0.11–0.53)

*OR, odds ratio; CI, confidence interval; Adjusted for sex, 5-year age class, residence, cigarette smoking, alcohol drinking, occupational physical activity, leisure-time physical activity, body mass index and parental history of colorectal cancer.

The risk of distal colon and rectal cancer was decreased in individuals with a history of colorectal polypectomy almost equally in the recent (<5 years) and distant (5+ years) past, but the risk of proximal colon cancer was rather elevated among those having undergone colorectal polypectomy 5+ years earlier. The latter finding may have been ascribed to chance, but needs consideration with respect to effectiveness of the current practice of colorectal endoscopy in Japan. Polyps detected at the distal segment generally warrant total colonoscopy in search for lesions in proximal sites of the colon (4). It is well documented that the presence of distal colorectal polyps, especially adenomas, is predictive of proximal colon neoplasms (24–27). It is thus possible that a portion of proximal colon polyps were undetected or excised incompletely. In fact, patients with colorectal adenomas or polyps removed during the period when sigmoidoscopy rather than colonoscopy was common, experienced a higher risk of colon or colorectal cancer compared with the general population (28,29).

In the present study, there was no measurable increase or decrease in the risk of overall or subsite-specific colorectal cancer in individuals with a history of colorectal polyps but not of colorectal polypectomy. However, because numbers of such persons were small in cases and controls, the findings need to be interpreted with caution. It is well known that individuals bearing colorectal polyps or adenomas have increased risk of colorectal cancer. For instance, patients with polyps who had not undergone polypectomy had an eight-fold increased risk of subsequent colorectal cancer as compared with general population in Osaka, Japan (14).

The fairly large size of the study, use of community controls and a high participation rate in the case group were strengths of the present study. There were several weaknesses to be discussed, however. In the present study, the participation rate was not as high in the controls as attained in 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 at most. Although a higher participation rate is of course desirable, the participation rate of 60% is considered to be acceptable (30). History of colorectal polyps and polypectomy was based on self-reporting. Individuals categorized as having no history of colorectal polyps may have had such lesions undiagnosed. The prevalence of reported colorectal polyps is influenced by previous participation in the screening for colorectal cancer and also by screening method. However, we did not elicit previous participation in the screening for colorectal cancer. It is another concern how representative the control subjects were with respect to the history of colorectal polyps and polypectomy. If individuals with a history of colorectal polypectomy had been more likely to participate in the study, the observed decrease in the risk associated with prior history of colorectal polypectomy would have been overestimated. Furthermore, case and control subjects may have recalled their past medical history differentially. However, it is unlikely that the cases recalled a prior history of colorectal polypectomy differentially according to different sites of colorectal cancer. Thus the present findings can not be totally ascribed to the so-recall bias.

In conclusion, in a large-scale case-control study of colorectal cancer, a prior history of colorectal polypectomy was associated with a substantial decrease in the risk of rectal cancer and possibly of distal colon cancer.

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Conflict of interest statement

None declared.

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Meat, fish and fat intake in relation to subsite-specific risk of colorectal cancer: The Fukuoka Colorectal Cancer Study

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High intake of red meat has been associated with increased risk of colorectal cancer in Western countries. There has been much interest in the role of n-3 polyunsaturated fatty acids (PUFA) in colorectal cancer prevention, but epidemiological findings are limited and inconsistent. The objective of our study was to examine associations of meat, fish and fat intake with risk of colorectal cancer, paying particular attention to the subsite within the colorectum. Data were from the Fukuoka Colorectal Cancer Study, a population-based case-control study, covering 782 cases and 793 controls. Diet was assessed by interview, using newly developed personal-computer software for registering semiquantitative food frequencies. The intake of beef/pork, processed meat, total fat, saturated fat or n-6 PUFA showed no clear association with the overall or subsite-specific risk of colorectal cancer. There was an almost significant inverse association between n-3 PUFA and the risk of colorectal cancer; the covariate-adjusted odds ratio for the highest (median 3.94 g/day) versus lowest (median 1.99 g/day) quintile of energy-adjusted intake was 0.74 (95% confidence interval 0.52–1.06, trend $P = 0.050$). The consumption of fish and fish products was similarly inversely related to the risk although the association was not statistically significant. These associations were more evident for distal colon cancer; adjusted odds ratio for the highest versus lowest quintile of n-3 PUFA intake was 0.56 (95% confidence interval 0.34–0.92, trend $P = 0.02$). Our findings do not support the hypothesis that consumption of red meat increases colorectal cancer risk but do suggest that high intake of fish may decrease the risk, particularly of distal colon cancer. (*Cancer Sci* 2007; 98: 590–597)

Colorectal cancer is one of the most common cancers in the world, accounting for nearly 10% of all incident cases.[1] In Japan, mortality from and incidence of colorectal cancer, especially of colon cancer, have increased markedly over the last decades,[2] and it has been argued that the increase is primarily due to Westernization of the Japanese diet.[3] Of the dietary factors possibly linked with colorectal cancer, fat intake has long been a matter of interest. Substantial data from animal and metabolic studies support a role for dietary fat in colorectal carcinogenesis, with high intake of saturated fat and n-6 polyunsaturated fatty acids (PUFA) documented to increase the incidence of chemically induced colon cancer in animal models.[4,5] However, results from case-control and cohort studies have consistently suggested a null association between total fat intake and colon or colorectal cancer, as reviewed elsewhere extensively.[6,7] It remains uncertain whether saturated or animal fat is related to increased risk of colorectal cancer.[6,7] However, high intake of red meat has been implicated as being associated with an increased risk of colon or colorectal cancer.[8,9]

There has been much interest recently in the role of n-3 PUFA and fish oil in colorectal cancer prevention. Studies in experimental animals have suggested that n-3 PUFA may be protective in colorectal carcinogenesis,[10] but epidemiological findings are limited and inconsistent. A protective association between fish consumption and colorectal cancer was observed in a cohort study of women in New York,[11] and a large cohort study in Europe,[12] but not in other cohort studies in Western countries as noted in a recent review.[13] A case-control study of French Canadians showed a clear protective association between dietary intake of n-3 PUFA and colorectal cancer,[14] whereas other case-control studies have generally failed to find a protective association with fish or n-3 PUFA intake in different countries.[15–17] In Japan, fish consumption was unrelated to colorectal cancer in two cohort studies,[18,19] but one of these found a decreased risk of colorectal cancer in individuals, especially in men, with high concentrations of serum n-3 PUFA.[20] A case-control study in Aichi Prefecture showed a decreased risk of colon cancer in men, but not in women, with high consumption of raw and cooked fish,[21] whereas a subsequent case-control study based on a revised questionnaire failed to find any association of fish and n-3 PUFA intake with either colon or rectal cancer.[22] Interestingly, the same research group recently reported an inverse association of docosahexaenoic acid, as well as of arachidonic acid and total PUFA, in erythrocytes with colorectal cancer in a case-control study.[23]

We here investigated associations of meat of different types, fish and individual types of fatty acids with colorectal cancer risk in the Fukuoka Colorectal Cancer Study.[24] Because these dietary factors may be differentially related to risks of cancer at different subsites of the colorectum,[25,26] we examined associations for the three subsites of the colorectum, that is, proximal colon, distal colon and rectum, separately.

Materials and Methods

The Fukuoka Colorectal Cancer Study is a population-based case-control study designed to examine the relationship of dietary

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