Genetic polymorphisms of *cyclooxygenase-2* and colorectal adenoma risk: The Self Defense Forces Health Study

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Cyclooxygenase (COX) is a key enzyme in the formation of prostaglandins, and an inducible isoform of COX, COX-2, has been implicated in colorectal carcinogenesis. This study investigated the relation of *COX-2* polymorphisms (–1195G>A, –765G>C and 8160A>G) to colorectal adenomas in a case–control study of male officials in the Self Defense Forces (SDF). The study subjects were 455 cases of colorectal adenoma and 1052 controls with no polyps who underwent total colonoscopy. Genotypes were determined using the polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method with genomic DNA extracted from the buffy coat. Statistical adjustment was made for age, hospital, rank in the SDF, body mass index (BMI), cigarette smoking, and alcohol intake. A statistically non-significant decrease in the risk of colorectal adenomas was observed for the AA versus GG genotype of –1195G>A polymorphism and for the GC versus GG genotype of –765G>C polymorphism. None had the –765CC genotype in either the case or control groups. No effect modification of overweight, smoking or alcohol use was observed for either –1195G>A or –765G>C polymorphism. The variant allele of the 8160A>G polymorphism was extremely rare. A haplotype of –1195G, –765G and 8160A alleles was associated with a modest increase in the risk (adjusted odds ratio [OR] 1.38, 95% confidence interval [CI] 0.99–1.91), and the increase was more evident for distal adenomas (adjusted OR 1.57, 95% CI 1.04–2.38). Another haplotype of –1195A, –765C and 8160A alleles showed an adjusted OR of 0.22 (95% CI 0.06–0.88). These findings add to evidence for the role of COX-2 in colorectal carcinogenesis and warrant further studies focusing on haplotypes. (*Cancer Sci* 2008; 99: 576–581)

COX is the key regulatory enzyme in the pathway of producing prostaglandins of different types from arachidonic acid. The enzyme has two isoforms, commonly referred to as COX-1 and COX-2.[1,2] COX-1 is expressed constitutively in most tissues, and prostaglandins derived from COX-1 play an important role in the maintenance of normal physiological homeostasis. COX-2 is normally absent in most cells and tissues, and is induced in response to inflammatory cytokines, hormones and growth factors.[1,2] COX-2 expression is enhanced in malignant or premalignant human tumors of various organs such as the colorectum, lung, breast, prostate, bladder, stomach, and esophagus.[3] Overexpression of COX-2 is linked to enhancement of cellular proliferation, promotion of angiogenesis, inhibition of apoptosis, stimulation of invasion, and suppression of immune responses.[2,3] Although COX-2 seems to be involved in carcinogenesis of various organs and tissues, much interest has been given to the role of COX-2 in colorectal carcinogenesis. Aspirin and other NSAIDs, known as COX inhibitors, have long been related to reduced risk of colorectal cancer in epidemiological studies,[4–7] as well as in animal experiments.[8,9] Randomized trials have shown that aspirin and celecoxib (a COX-2 inhibitor), reduce the recurrence of colorectal adenomas in patients with previous colorectal adenomas and cancer.[10–14]

A large number of polymorphisms have been identified in the *COX-2* gene, but little has been investigated regarding their functionality. The *COX-2*–765G>C polymorphism (dbSNP ID: rs204179) has been described as related to an approximately 30% decrease in promoter activity, probably by disrupting the binding site of stimulatory protein 1, and patients harboring the –765C allele have showed a lower level of inflammation after coronary artery bypass surgery, compared with those homozygous for the –765G allele.[15] While the *COX-2*–765G>C polymorphism was not related to risk of colorectal cancer,[16–18] individuals homozygous for the –765C allele tended to have a lowered risk of colorectal adenomas and hyperplastic polyps.[19] In contrast, the *COX-2*8160A>G polymorphism (dbSNP ID: rs4648298) located in the untranslated region of exon 10 has been associated with an increased risk of colorectal cancer,[17] but the function of this polymorphism is unknown. It was recently reported that the –1195G>A polymorphism (dbSNP ID: rs6894669) affected the transcription activity of the *COX-2* gene with a higher promoter activity for the –1195A allele.[20] Individuals homozygous for the –1195A allele had increased risks of esophageal squamous cell carcinoma,[20] and gastric cancer,[21] and an increased risk of esophageal cancer was also observed for those heterozygous for the –1195A allele.[20] In the present study, we examined the relation of these three genetic polymorphisms of the *COX-2* gene to colorectal adenomas in middle-aged Japanese men.

Materials and Methods

Subjects. Study subjects were male officials in the SDF who received a pre-retirement health examination at the SDF Fukuoka Hospital or Kumamoto Hospital during the period from January 1997 to March 2001. The pre-retirement health examination was a nationwide program offering a comprehensive medical examination to those retiring from the SDF. Details of the pre-retirement health examination have been described elsewhere.[22–24] A sample of 7 mL of fasting venous blood was donated for the purpose of medical research with written informed consent. The study was approved by the ethics committee of Kyushu University Faculty of Medical Sciences.

The present study included 455 cases of histologically confirmed colorectal adenoma and 1052 controls with no polyps who underwent total colonoscopy. In the consecutive series of

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Abbreviations: BMI, body mass index; COX, cyclooxygenase; CI, confidence interval; NSAID, non-steroidal anti-inflammatory drug; OR, odds ratio; PCR-RFLP, polymerase chain reaction–restriction fragment length polymorphism; SDF, Self Defense Forces.

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2454 men, except for five men who refused to participate in the survey, we excluded 77 men who did not receive colonoscopy and 242 men who had a prior history of colectomy ($n = 17$), colorectal polypectomy ($n = 212$), malignant neoplasm ($n = 27$) or inflammatory bowel disease ($n = 1$). In the remaining 2135 men, colonoscopic findings were classified as colorectal cancer ($n = 1$), polyp ($n = 938$), non-polyp benign lesion such as diverticula ($n = 123$) and normal ($n = 1073$). Of the 938 with colorectal polyps, 461 were found to have adenoma without *in situ* or invasive carcinoma. Of the 1196 with normal colonoscopy or non-polyp benign lesions, 1067 underwent total colonoscopy and were used as controls. DNA was not available for six cases and 11 controls, and 455 cases and 1052 controls remained in the analysis. The numbers of cases having adenomas at the proximal colon alone, the distal colon and/or rectum alone and both proximal and distal segments were 149, 239 and 67, respectively. The proximal colon included the cecum, ascending colon and transverse colon. The cases with rectal adenomas alone numbered only 42, and distal colon and rectal adenomas were combined.

Genotyping. DNA was extracted from the buffy coat using a commercial kit (QIAGEN, Hilden, Germany). Genotyping was carried out using the PCR-RFLP method with electrophoresis on agarose gels and visualization using ethidium bromide. The PCR was carried out in a reaction mixture of 10 μL containing 0.5 U *Taq* and 1 μL template DNA with a concentration of approximately 50–150 ng/μL. The *COX2*–1195G>A polymorphism was determined according to the method described by Zhang *et al.*[20] using primers 5′-CCC TGA GCA CTA CCC ATG AT-3′ (sense) and 5′-GCC CTT CAT AGG AGA TAC TGG-3′ (antisense). The PCR product of 273 bp was digested with *Pvu*II into 220 bp and 53 bp fragments for the –1195G allele. The –765G>C polymorphism was determined, as described by Papafili *et al.*,[15] using primers 5′-CCG CTT CCT TTG TCC ATC AG-3′ (sense) and 5′-GGC TGT ATA TCT GCT CTA TAT GC-3′ (antisense). The restriction enzyme *Aci*I digested the 306-bp PCR product into two fragments of 188 bp and 118 bp for the –765C allele. The 8160A>G polymorphism was determined, as described by Cox *et al.*,[18] using primers 5′-CGT TCC CAT TCT AAT TAA TGC CCT T-3′ (sense) and 5′-ATT AAA ACC CAC AGT GCT TGA CAC A-3′ (antisense). The restriction enzyme *Alu*I digested the 545-bp PCR product into 349 bp and 196 bp fragments in the case of the rare 8160G allele.

Lifestyle questionnaire. A self-administered questionnaire was used to ascertain smoking habits, alcohol consumption and other lifestyle factors prior to colonoscopy. Methods of ascertaining lifestyle factors have previously been described in detail. Cumulative exposure to cigarette smoking was expressed as cigarette-years, which were calculated by multiplying the average number of cigarettes per day by the total years of smoking. Cigarette smoking was classified into 0, 1–399, 400–799 and ≥800 cigarette-years. Daily intake of ethanol was estimated for current alcohol drinkers based on consumption frequencies and amounts of five types of alcoholic beverages on average in the past year, and alcohol use was categorized into never, past and current use with a consumption of <30, 30–59 or ≥60 mL of ethanol per day. BMI was calculated by dividing weight in kilograms by squared height in meters, and was categorized into four levels using quartiles in the distribution in the control group.

Statistical analysis. The association between the *COX2* polymorphism and colorectal adenoma was assessed by means of OR and 95% CI, which were derived from logistic regression analysis. Statistical adjustment was made for age (continuous variable), hospital, rank of the SDF (low, intermediate and high), cigarette smoking, alcohol use and BMI. Statistical significance was declared if a two-sided P-value was <0.05 or if the 95% CI did not include unity.

The Hardy–Weinberg equilibrium of the genotype distribution was tested using the chi-squared test with a degree of freedom of one, and the pair-wise linkage disequilibrium was evaluated using Lewontin's D' for the biallelic markers. Haplotypes were estimated using the expectation–maximization algorithm to generate maximum likelihood estimates of haplotype frequencies. Individuals were given the probability of having a specific pair of haplotypes, and the OR for a specific haplotype was obtained using logistic regression analysis with each haplotype as an independent variable. SAS/Genetics version 9.1 and SAS/Statistics version 8.2 were used (SAS Institute, Cary, NC, USA).

Results

The characteristics of colorectal adenoma cases and controls are summarized in Table 1. The age range was 50–57 years in cases and 47–59 years in controls, with the same mean age in the two groups. High exposure to cigarette smoking and high alcohol

Table 1. Characteristics of cases and controls

Variable	Cases ($n = 455$)	Controls ($n = 1052$)	P-value*
Age (years), mean (SD)	52.4 (0.8)	52.4 (0.9)	0.80
Hospital (%)			0.30
Fukuoka	71.4	68.7	
Kumamoto	28.6	31.3	
Rank (%)			0.77
Low	60.4	62.0	
Intermediate	25.3	23.6	
High	14.3	14.4	
Cigarette-years (%)			<0.0001
0	20.9	33.8	
1–399	14.1	18.9	
400–799	45.5	33.7	
≥800	19.6	13.6	
Alcohol use (%)			0.0003
None	11.2	13.7	
Past	2.9	3.2	
<30 (mL/day)	21.1	30.7	
30–59	34.1	28.7	
≥60	30.8	23.7	
Body mass index (kg/m^2), mean (SD)	24.1 (2.8)	23.7 (2.5)	0.01

*Based on a t-test for the comparison of means and a chi-squared test for the comparison of frequency distribution. SD, standard deviation.

consumption were more frequent in the cases than in the controls. BMI was also greater in the cases.

The –1195G>A polymorphism was fairly common, with the –1195A allele at a frequency of 53.1% in the control group. In contrast, the –765C allele was relatively rare, and there was no individual homozygous for the –765C allele in either the case or control group. The variant allele of the 8160A>G polymorphism was extremely rare, and even those with the heterozygous genotype were very few (Table 2). The frequency of the –765C and 8160G alleles in the controls was 2.9% and 0.4%, respectively. The genotype distribution of the –1195G>A, –765G>C and 8160A>G polymorphisms each did not deviate from the Hardy–Weinberg equilibrium

The AA genotype of the –1195G>A polymorphism was slightly less frequent in the cases than in the controls, and the OR for the AA versus GG genotype was lower than unity; decreases in the crude and adjusted OR were short of statistical significance ($P = 0.058$ and $P = 0.069$, respectively). The GC genotype of the –765G>C polymorphism was also less frequent in the case group, and the crude OR for the GC versus GG genotype was significantly lower than unity ($P = 0.038$). Adjustment for the covariates did not much change the OR, but the decrease failed to reach statistical significance ($P = 0.057$). No association was observed between the 8160A>G polymorphism and colorectal adenomas. Decreases in the OR associated with the –1195AA and –765GC genotypes were slightly more pronounced for distal adenomas. The adjusted OR of proximal adenomas for the GA and AA genotypes versus the GG genotype of the –1195G>A polymorphism was 0.95 (95% CI 0.61–1.47) and 0.78 (95% CI 0.47–1.29), respectively, while the corresponding OR of distal adenomas was 0.90 (95% CI 0.63–1.27) and 0.64 (95% CI 0.42–0.98), respectively. As for the –765G>C polymorphism, the adjusted OR of proximal and distal adenomas for the GC versus GG genotype was 0.92 (95% CI 0.453–2.00) and 0.51 (95% CI 0.23–1.15), respectively.

In the multivariate model without variables for the genetic polymorphism, evident increases in the adjusted OR were observed for the highest category of BMI (≥25.2 kg/m^2), for the highest two categories of cigarette smoking (≥400 cigarette-years) and for the highest two categories of alcohol consumption (≥30 mL/day). Thus the analysis of effect modification of these lifestyle factors was based on dichotomous variables of overweight (BMI ≥ 25.0 kg/m^2), high exposure to cigarette smoking (≥400 cigarette-years) and high alcohol consumption (≥30 mL/day). Neither the –1195G>A nor the –765G>C polymorphism showed any measurable interaction with respect to overweight (–1195G>A: $P = 0.48$; –765G>C: $P = 0.58$), cigarette smoking (–1195G>A: $P = 0.83$; –765G>C: $P = 0.62$) or alcohol use (–1195G>A: $P = 0.68$; –765G>C: $P = 0.45$). The site-specific analysis also showed no clear effect modification of these covariates on the association with either the –1195G>A or the –765G>C polymorphism (data not shown).

Lewontin's D' was 0.94 for the linkage disequilibrium of –1195G>A and –765G>C, and each of the other pairs naturally showed a complete linkage disequilibrium. The frequency of the estimated haplotypes for cases and controls is shown in Table 3. The two predominant haplotypes, that is, the combination of –1195A, –765G and 8160A (haplotype A-G-A) and that of –1195G, –765G and 8160A (haplotype G-G-A), accounted for 97% of the possible haplotypes in all subjects. Although the overall distribution of the estimated haplotypes did not show a statistically

Table 2. *COX-2* polymorphisms and risk of colorectal adenomas

Genotype	Numbers (%)		Odds ratio (95% confidence interval)	
	Case	Control	Crude	Adjusted†
–1195G>A				
GG	106 (23.3)	227 (21.6)	1.00 (referent)	1.00 (referent)
GA	249 (54.7)	532 (50.6)	1.00 (0.76–1.32)	1.02 (0.77–1.35)
AA	100 (22.0)	293 (27.9)	0.73 (0.53–1.01)	0.74 (0.54–1.02)
–765G>C‡				
GG	440 (96.7)	989 (94.0)	1.00 (referent)	1.00 (referent)
GC	15 (3.3)	62 (5.9)	0.54 (0.31–0.97)	0.57 (0.31–1.02)
CC	0	0	–	–
8160A>G‡				
AA	451 (99.1)	1042 (99.1)	1.00 (referent)	1.00 (referent)
AG	4 (0.9)	9 (0.9)	1.03 (0.31–3.35)	1.24 (0.37–4.21)
GG	0	0	–	–

†Adjusted for age, hospital, rank, body mass index, cigarettes smoking and alcohol use. ‡Genotypes were not determined for one control.

Table 3. Estimated frequencies of *COX-2* haplotypes and odds ratios of colorectal adenomas for each haplotype

Haplotype†	Case	Haplotype frequency		Odds ratio (95% confidence interval)	
		Control	*P*-value*	Crude	Adjusted‡
G-G-A	0.50656	0.46691	0.045	1.39 (1.01–1.91)	1.38 (0.99–1.91)
A-G-A	0.47695	0.50359	0.179	0.80 (0.59–1.11)	0.80 (0.58–1.11)
A-C-A	0.01206	0.02400	0.033	0.21 (0.06–0.83)	0.22 (0.06–0.88)
A-C-G	0.00440	0.00428	0.965	1.05 (0.10–11.2)	1.54 (0.13–17.8)
G-C-A	0.00003	0.00121	0.311	–§	–§
G-C-G	0.00000	0.00000	0.932	–§	–§

*Based on a chi-squared test with one degree of freedom, comparing frequencies of a specified haplotype and all other haplotypes combined. †Sequence of –1195G>A, –765G>C and 8160A>G. ‡Adjusted for age, hospital, rank, body mass index, cigarettes smoking and alcohol use. §Not presented because of extremely unstable estimates.

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significant difference between cases and controls ($P = 0.26$), the combination of –1195G, –765G and 8160A (haplotype G-G-A) was more frequent in the cases with the statistical significance ($P = 0.045$), and the combination of –1195A, –765C and 8160A (haplotype A-C-A) was twice less frequent in the cases ($P = 0.033$). Statistically significant decreases were noted for both crude and adjusted OR in relation to haplotype A-C-A. A modest increase in the OR was noted for haplotype G-G-A; the crude OR was statistically significantly greater than unity, and the adjusted OR was just short of statistical significance ($P = 0.054$). An increase in the risk associated with haplotype G-G-A was greater for distal adenomas than for proximal adenomas; the adjusted OR was 1.29 (95% CI 0.79–2.13) for proximal adenomas and 1.57 (95% CI 1.04–2.38) for distal adenomas. The adjusted OR of proximal and distal adenomas for haplotype A-C-A was 0.40 (95% CI 0.06–2.81) and 0.27 (95% CI 0.04–1.58), respectively.

Finally, the OR for the two haplotypes was examined in the low and high categories of BMI, smoking and alcohol use each (Table 4). An increase in the OR of adenomas, particularly at the distal segment, associated with haplotype G-G-A seemed to be limited to overweight men, although the interaction was not statistically significant. Decreases in the OR of colorectal adenomas associated with haplotype A-C-A did not much vary with categories of the covariates. We refrained from stratified analysis for proximal adenomas because the number was small.

Discussion

The present study showed a decreased risk of colorectal adenomas for the *COX-2*–1195AA genotype compared with the –1195GG and for the –765GC genotype versus –765GG genotype in a Japanese population. The 8160A>G polymorphism was found to be extremely rare in Japanese, and the present study did not provide useful information regarding the role of this polymorphism in colorectal carcinogenesis. The haplotype analysis indicated that a common combination of –1195G, –765G and 8160A alleles was associated with a modest increase in the risk of colorectal adenomas, while a rare combination of –1195A, –765C and 8160A alleles was related to a fairly large decrease in the risk. It is notable that the increased risk associated with the former haplotype was more evident for distal adenomas.

In a previous colonoscopy-based study in the USA,[19] the –765CC genotype was associated with decreased risk of colorectal adenomas and hyperplastic polyps, particularly among those not using NSAIDs, whereas there was no decrease in the risk of either adenomas or hyperplastic polyps in individuals heterozygous for the variant allele (–765GC genotype). Regarding the –765G>C polymorphism and colorectal cancer, there was no difference in the genotype distribution between 148 cases and 241 controls in Japan,[16] between 310 cases and 1177 controls in Singapore Chinese,[17] and between 292 cases and 274 controls in Spain.[18] In the study of Chinese in Singapore,[17] individuals with the –765GC or –765CC genotype showed a statistically significant two-fold increase in the risk of colon cancer, but not of rectal cancer, when they had high dietary intake of n-6 polyunsaturated fatty acids. In that study, however, only 25 cases of colon cancer and 12 cases of rectal cancer had the –765C allele, and the cases were much fewer in further stratification by the dietary factor. Caution is thus needed in the interpretation of results from such a small study.

Recent studies have lent further support to the initial observation that the –765C allele was associated with suppression of inflammation.[15] Individuals carrying the –765C allele had lower risks of myocardial infarction and ischemic stroke in Italy,[25] and the –765C allele has been associated with lower COX-2 expression and reduced atherosclerosis in patients with hypercholesterolemia in Spain.[26] It was also reported in the USA that individuals with the –765C allele had a decreased risk of Alzheimer's disease.[27] In contrast, the –765C allele has been associated with an increased risk of gastric cancer in Portugal.[28] In China, the –765GC genotype has been associated with an increased risk of esophageal cancer,[20] but not of gastric cancer,[21] compared with the –765GG genotype. The –765C allele is fairly common in Caucasians and African Americans, with frequencies of 25–50% in different countries,[28] while the variant allele is relatively rare in Asians. The frequency of the –765C allele in the control subjects

Table 4. Haplotype-specific risk of colorectal adenomas in low and high categories of body mass index (BMI), smoking and alcohol use

Covariate	Category	Adjusted odds ratio (95% confidence interval)†	
		Haplotype G-G-A‡	Haplotype A-C-A‡
Adenomas of all sites			
BMI (kg/m^2)	<25.0	1.18 (0.79–1.76)	0.26 (0.06–1.24)
	≥25.0	1.74 (0.97–3.13)	0.13 (0.01–3.16)
	Interaction	$P = 0.25$	$P = 0.62$
Cigarette-years	<400	1.56 (0.91–2.67)	0.19 (0.01–2.36)
	≥400	1.30 (0.85–1.98)	0.23 (0.04–1.24)
	Interaction	$P = 0.84$	$P = 0.88$
Alcohol (mL/day)	<30	1.21 (0.72–2.03)	0.49 (0.07–3.22)
	≥30	1.54 (1.00–2.36)	0.11 (0.01–0.81)
		$P = 0.51$	$P = 0.26$
Distal adenomas			
BMI (kg/m^2)	<25.0	1.29 (0.77–2.14)	0.30 (0.04–2.15)
	≥25.0	2.14 (1.03–4.44)	0.11 (0.00–8.60)
	Interaction	$P = 0.23$	$P = 0.68$
Cigarette-years	<400	1.91 (0.96–3.81)	0.29 (0.01–6.14)
	≥400	1.44 (0.85–2.45)	0.23 (0.03–2.10)
	Interaction	$P = 0.65$	$P = 0.90$
Alcohol (mL/day)	<30	1.52 (0.77–3.01)	0.25 (0.01–5.09)
	≥30	1.64 (0.97–2.78)	0.26 (0.03–2.42)
	Interaction	$P = 0.94$	$P = 1.00$

†Adjusted for age, hospital, rank and the two other listed covariates. ‡Sequence of –1195G>A, –765G>C and 8160A>G.

(2.9%) was similar to the frequencies reported previously in Japan (2.3%),[16] and China (2.2%),[20] but slightly less frequent than that observed in Chinese in Singapore (4.8%).[17]

The present study was the first that addressed the role of the *COX-2* –1195G>A polymorphism in colorectal carcinogenesis. The decreased risk of colorectal adenomas associated with the –1195AA genotype was rather unexpected, because the –1195AA genotype has been shown to be related to increased risk of esophageal and gastric cancer in China.[20,21] The frequency of the –1195A allele was approximately 51% in China,[20] and the allele frequency was similar in the present study (53%). Further studies are needed to consolidate a protective association between the –1195G>A polymorphism and colorectal cancer or adenomas. It is possible that the –1195G>A polymorphism may be differently associated with different sites of cancer.

We examined the relation between the 8160A>G polymorphism and colorectal adenomas because this polymorphism has been reported to be associated with a 2.5-fold increase in the risk of colorectal cancer in a case–control study in Spain.[18] The functionality of this polymorphism is unknown, but it is speculated that the polymorphism may be linked to stability of mRNA because the polymorphism is located very near to the first poly A signal.[18] The variant allele of the Val511Ala polymorphism (5939T>C, dbSNP ID: rs5273) has been reported to be related to a decreased risk of colorectal cancer and adenomas in African Americans,[29,30] but this polymorphism is not seen in other ethnic populations, including Japanese.[16] The distribution of *COX-2* polymorphisms seems to differ substantially by ethnicity, and different *COX-2* polymorphisms are probably related to colorectal cancer and adenomas in different populations.

Overweight, smoking and alcohol use did not measurably modify the association with either –1195G>A or –765G>C polymorphism. Interestingly, a suggestive interaction was noted for haplotype G-G-A and overweight, particularly in relation to the risk of distal adenomas, while the decreased risk associated with haplotype A-C-A did not differ by BMI and the other covariates. Careful interpretation is needed for the findings from a subgroup analysis, but further studies are warranted.

The present study had methodological advantages in that colonoscopy was carried out non-selectively in a defined population and in that the absence of polyp lesions was confirmed in the control subjects using total colonoscopy. The study subjects were not representative of Japanese men in the general population, but selection was unlikely to exist with regard to the genetic polymorphisms under study, as discussed above. The use of aspirin and other NSAIDs may have exerted an effect modification, as suggested in the previous study of colorectal polyps.[20] The lack of information regarding use of NSAIDs was a weakness, but regular users of NSAIDs were probably few in the present study population. Dietary factors were not taken into account because validated data were not available for specific foods and nutrients. Of particular interest is the effect modification of *n*-6 and *n*-3 polyunsaturated fatty acids because the balance of these fatty acids in cell membranes modulates the production of arachidonic acid-derived eicosanoids.

In conclusion, a case–control study suggested decreased risks of colorectal adenoma associated with –1195G>A and –765G>C polymorphisms in a population of Japanese men. Two haplotypes related to the risk of colorectal adenomas were identified, and an increased risk associated with one of the haplotypes was more evident for distal adenomas, particularly among overweight men. The findings add to evidence for the role of COX-2 in colorectal carcinogenesis and warrant further studies focusing on haplotypes.

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