Red Meat Intake, *CYP2E1* Genetic Polymorphisms, and Colorectal Cancer Risk[1]

Loïc Le Marchand,[2] Timothy Donlon, Ann Seifried, and Lynne R. Wilkens

Etiology Program, Cancer Research Center of Hawaii, University of Hawaii, Honolulu, Hawaii 96813

Abstract

***N*-Nitroso compounds are suspected colorectal cancer (CRC) carcinogens to which individuals on a diet high in red meat (RM) may be particularly exposed. Many of these compounds undergo α-hydroxylation by CYP2E1 to form DNA adducts. The gene coding for this enzyme is polymorphic and thus may constitute a susceptibility factor for CRC. We conducted a population-based case-control study in Hawaii to test the association of two functional polymorphisms in *CYP2E1* (the G1259C *Rsa*I substitution and a 5′ 96-bp insertion variant) with CRC, as well as their modifying effects on the association of RM and processed meat (PM) with this cancer. We obtained interviews and blood samples for 521 patients with CRC (165 with rectal cancer) and 639 controls of Japanese, Caucasian, or Hawaiian origin. Genotyping was performed by PCR. After adjustment for CRC risk factors, subjects with the 5′ insert variant were found to be at a 60% increased risk (95% confidence interval, 1.1–2.5) for rectal cancer. Subjects who carry the insert and who were predicted to have been exposed to increased levels of nitrosamines, based on their high intake of RM or PM, were at a markedly greater increased risk (2- and 3-fold for RM and PM, respectively) for rectal cancer. No clear association was found for colon cancer. A similar increase in rectal cancer risk was found for *CYP2E1* insert carriers who consumed salted/dried fish or Oriental pickled vegetables. These data provide additional support for the hypothesis that nitrosamines are carcinogenic to the rectum in humans and that RM and, in particular, PMs are significant sources of exposure for these compounds.**

Introduction

Recent evaluations of the diet and CRC[3] literature have concluded that high RM and PM intakes are probable risk factors for this disease (1, 2). These foods may be a source of exposure to chemical carcinogens, such as HAAs or PAHs that are formed when meat is cooked at high temperature and/or on an open flame (3). Exposure to NOCs may also be increased on a high-RM diet because these compounds are formed in the digestive tract from the reaction of amines with nitrite contained in cured meat (4) or generated by the colonic flora (5, 6). In contrast to PAHs and HAAs, the possible etiological role of NOCs in CRC has received only limited attention (4, 6).

Nitrosamines require metabolic activation by cytochrome P450 enzymes before they can bind to DNA, initiating the carcinogenic process. CYP2E1 is a key activating enzyme because it catalyzes the α-hydroxylation of many nitrosamines (7). This enzyme is predominantly expressed in the liver and is induced or inhibited by several chemicals, hormones, or metabolic conditions (8). Its activity shows significant interindividual variation due in part to inherited alterations of the structural gene. A substitution polymorphism (G1259C) detected using the restriction enzymes *Pst*I or *Rsa*I has been associated with a decreased CYP2E1 activity/inducibility (8, 9). More recently, a 96-bp insertion polymorphism in the regulatory region of *CYP2E1* has been associated with an increased induction by obesity or ethanol (10). We hypothesized that if NOCs play a role in CRC, and if RM and PMs are significant sources of exposure for these compounds, the aforementioned polymorphisms should be associated with this disease and should modify the association of these foods with CRC. We tested this hypothesis in a large case-control study investigating gene-diet interactions and CRC in Hawaii.

Materials and Methods

The methodology for this study has been described previously (11). In short, cases were identified through the Hawaii Tumor Registry, a member of the United States National Cancer Institute's Surveillance, Epidemiology, and End Results program. Eligible cases were all Oahu residents diagnosed before age 85 years with a primary adenocarcinoma of the colon or rectum between January 1994 and August 1998. Only patients who were at least 75% Japanese or Caucasian or had any percentage of Hawaiian ancestry were included. Controls were selected from participants in an ongoing health survey conducted by the Hawaii State Department of Health among a 2% annual random sample of the state households. This source was supplemented with controls 65 years or older from Health Care Financing Administration participants on Oahu. One control was matched to each case on sex, ethnicity, and age. Personal interviews were obtained from 768 matched pairs of cases and controls, resulting in a participation rate of 58.2% and 53.2% for cases and controls, respectively. Compared with noninterviewed cases, interviewed cases had a similar ethnic distribution, were less likely to have a regional or distant metastasis (46% *versus* 55%), and were younger by an average of 1.8 years. A blood sample was obtained for 548 cases (71.3% of interviewed

Received 9/28/01; revised 5/31/02; accepted 6/14/02.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] Supported in part by Grant R01-CA60987 and Contract N01-CN55424 from the National Cancer Institute, United States Department of Health and Human Services.
[2] To whom requests for reprints should be addressed, at Etiology Program, Cancer Research Center of Hawaii, University of Hawaii, 1236 Lauhala Street, Suite 407, Honolulu, HI 96813.
[3] The abbreviations used are: CRC, colorectal cancer; OR, odds ratio; CI, confidence interval; RM, red meat; PM, processed meat; HAA, heterocyclic amine; PAH, polycyclic hydrocarbon; NOC, *N*-nitroso compound.

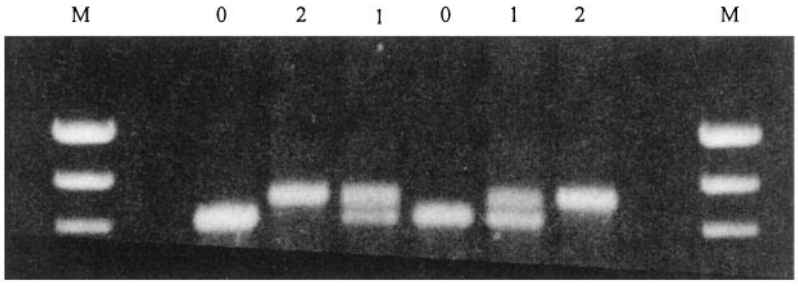

Fig. 1. Representative gel showing the three genotypes for the *CYP2E1* insert. *Lanes 0* show subjects with no insert. The product is 852-bp long. *Lanes 2* show individuals with two inserts. The product is 948-bp long. *Lanes 1* show individuals heterozygous for the insert with the 852- and 948-bp products. *Lanes M*, a pGEM DNA size marker (Promega).

cases) and 656 controls (85.4% of interviewed controls). Cases and controls who donated blood were similar to all interviewed subjects with regard to age, sex, and ethnicity. DNA stock was depleted for 27 cases and 17 controls.

In-person interviews were conducted at the subjects' homes by trained interviewers within 4.5 months of diagnosis, on average. The questionnaire included detailed information on demographics, including the race of each grandparent; a quantitative food frequency questionnaire; a lifetime history of tobacco, alcohol, and aspirin use; a history of recreational sports activities since age 18 years; and a family history of CRC in parents and siblings. The diet questionnaire has been described previously and validated in this population (12). Frequencies and amounts consumed were obtained for 268 food items or categories. Participants reported their average frequencies of consumption and average portion sizes for those items eaten at least 12 times a year during the year before onset of symptoms for cases and during the previous 12 months for controls. A food composition nutrient database, based primarily on the United States Department of Agriculture's nutrient database and supplemented from other sources, was applied to compute nutrient intakes from each food item. Daily intakes for nutrients were summed across food items to obtain daily amounts for each individual. Daily intakes of food groups (*e.g.*, RM) were also computed by summing the gram intakes across relevant food items or the appropriate portion of mixed dishes. Participants also reported their frequencies and dosages of vitamin and mineral supplement intake during the reference period.

The genotyping for the *CYP2E1* polymorphisms was performed blinded to case-control status. Our assay for the *Rsa*I polymorphism was described previously (8). Genotyping for the 96-bp insertion in the 5′-flanking region of *CYP2E1* described by MacCarver *et al.* (10) was carried out by PCR. The primers used were 5′-TCACTCCATGAACGAGTTGG-3′ and 5′-GGTGAGAACAGGAAGCATCAG-3′. The PCR conditions consisted of an initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 61°C for 30 s, and 72°C for 1 min 30 s, with a final extension at 72°C for 10 min. The PCR product was subjected to electrophoresis on 1% SeaKem ME agarose gel. The product is 852-bp long, and the insertion gives a product that is 96-bp longer (Fig. 1).

The statistical analysis used unconditional logistic regression (13). The models were adjusted for the study matching variables (age, sex, and ethnicity) and for the covariates found to be associated with CRC in this study, namely, pack-years of cigarette smoking, lifetime recreational physical activity (hours), body mass index 5 years ago, lifetime use of aspirin (months), years of schooling, and intakes of non-starch polysaccharides from vegetables and calcium from foods and supplements (11). All of the covariates were entered as continuous variables. Intakes of nutrients were adjusted for caloric intake by the method of residuals (14). Because the ORs for the exposures of interest were similar in men and women, results are presented for both sexes combined. Genotype was modeled by indicator variables and by a trend variable assigned the number of variant alleles. The likelihood ratio test was used to test for interaction among variables with respect to CRC. The test compared a main effects, no interaction model with a fully parameterized model containing all possible interaction terms for the variables of interest.

Results

The characteristics of the subjects have been described previously (11). Sixty percent were Japanese, 26% were Caucasian, and 14% were Native Hawaiian. The frequency for the *Rsa*I variant allele among controls was 23.2% in Japanese, 4.1% in Caucasians, and 14.8% in Native Hawaiians. The corresponding allele frequencies for the 5′ insert were 22.7%, 2.0%, and 9.2%. These frequencies are similar to those available from previous reports (8, 10). Table 1 shows the adjusted ORs for CRC by *CYP2E1* genotype and subsite of the large bowel. A weak inverse association was suggested for the *Rsa*I low activity (variant) genotype for both colon and rectal cancers. The OR for subjects with at least one variant *Rsa*I allele was 0.8 (95% CI, 0.6–1.1) and 0.8 (95% CI, 0.6–1.3) for colon and rectal cancer, respectively. A statistically significant 60% increase in risk of rectal cancer (95% CI, 1.1–2.5) was observed for subjects with the insert variant, compared with those with the homozygous wild-type genotype. The insert polymorphism was not associated with colon cancer. ORs were also computed for right-sided and left-sided colon cancer. No association was found with either *CYP2E1* polymorphism.

Table 2 presents the joint effects of meat intake and the *CYP2E1* polymorphisms on CRC risk. For colon cancer, RM (without PMs) was not associated with risk, whereas a weak direct association was observed for PMs. There was little evidence of a modifying effect of *CYP2E1* on these relationships. For rectal cancer, a statistically significant increased risk was observed for subjects with both a high intake of RM or PM and the insert polymorphism. Rectal cancer risk for subjects with the insert variant and a RM consumption over the median was 2.1 (95% CI, 1.2–3.7), compared with those with no insert and a RM intake $\leq$ median. For PMs, this effect was more marked, with a rectal cancer OR of 3.1 (95% CI, 1.8–5.6) for subjects with the insert and a high meat intake. However, none of the tests for interaction was statistically significant. Interactions were also examined between *CYP2E1* and ethanol intake and body mass. No suggestion of interaction was detected. A variable was also created for each subject by taking the sum of the number of high activity alleles for each of the two polymorphisms. This variable yielded risk estimates that were similar to those found for the insertion polymorphism.

Table 3 presents the joint effects on CRC risk of the two

Table 1 CRC ORs[a] and CIs for the *CYP2E1 Rsa*I and insert polymorphisms

CYP2E1		All		Colon		Rectum	
*Rsa*I	*c1/c1*	384/449[b]	1.0	271/449	1.0	120/449	1.0
	c1/c2	116/164	0.8 (0.6–1.1)	78/164	0.8 (0.6–1.1)	42/164	0.9 (0.6–1.3)
	c2/c2	21/26	0.9 (0.5–1.6)	19/26	1.2 (0.6–2.2)	3/26	0.4 (0.1–1.3)
			$P = 0.20$[c]		$P = 0.48$		$P = 0.22$
	any *c2*	137/190	0.8 (0.6–1.1)	97/190	0.8 (0.6–1.1)	45/190	0.8 (0.6–1.3)
Insert	*0*	357/468	1.0	261/468	1.0	104/468	1.0
	1	133/137	1.3 (1.0–1.8)	88/137	1.2 (0.9–1.7)	49/137	1.7 (1.1–2.6)
	2	21/32	0.9 (0.5–1.6)	12/32	0.7 (0.3–1.2)	9/32	1.4 (0.6–3.1)
			$P = 0.34$		$P = 0.99$		$P = 0.55$
	1 or *2*	154/169	1.3 (0.9–1.7)	100/169	1.1 (0.8–1.5)	58/169	1.6 (1.1–2.5)

[a] Adjusted by unconditional logistic regression for age, sex, ethnicity, pack-years of cigarette smoking, lifetime recreational physical activity (hours), lifetime aspirin use (months), body mass index 5 years ago, years of schooling, and intakes of nonstarch polysaccharides from vegetables and calcium from foods and supplements.
[b] No. of cases/no. of controls. The *CYP2E1* insert genotype was missing for 10 cases and 2 controls for whom DNA was depleted. Twelve cases had multiple colorectal tumors involving both colon and rectum, and these cases were included in both subsite models.
[c] *P* for gene-dosage effect.

Table 2 Joint effects of *CYP2E1* and RM and PM intakes on CRC risk

Subsite	*CYP2E1*		RM (g/day) (without processed meats)[a]					PMs (g/day)[b]				
			≤Median		>Median		*P*[c]	≤Median		>Median		*P*
			n[c]	OR[d] (95% CI)	*n*	OR (95% CI)		*n*	OR (95% CI)	*n*	OR (95% CI)	
Colon	*Rsa*I	*c1/c1*	118/217	1.0	153/232	1.1 (0.8–1.5)		107/221	1.0	164/228	1.4 (1.0–1.9)	
		Any *c2*	56/98	1.1 (0.7–1.6)	41/92	0.8 (0.5–1.2)	0.18	43/102	0.9 (0.6–1.4)	54/88	1.2 (0.6–1.4)	0.81
	Insert	*0*	126/229	1.0	135/239	0.9 (0.7–1.3)		101/234	1.0	160/234	1.4 (1.0–2.0)	
		1 or *2*	43/86	0.9 (0.6–1.4)	57/83	1.1 (0.7–1.7)	0.28	43/87	1.1 (0.7–1.8)	57/82	1.5 (0.9–2.3)	0.79
Rectum	*Rsa*I	*c1/c1*	44/217	1.0	76/232	1.3 (0.8–2.0)		38/221	1.0	82/228	1.7 (1.0–2.6)	
		Any *c2*	21/98	1.0 (0.5–1.8)	24/92	1.1 (0.6–1.9)	0.64	14/102	0.8 (0.4–1.6)	31/88	1.6 (0.9–2.9)	0.66
	Insert	*0*	44/229	1.0	60/239	1.1 (0.7–1.7)		35/234	1.0	69/234	1.5 (0.9–2.4)	
		1 or *2*	20/86	1.3 (0.7–2.5)	38/83	2.1 (1.2–3.7)	0.30	16/87	1.3 (0.6–2.5)	42/82	3.1 (1.8–5.6)	0.22

[a] All beef, pork, veal, and lamb items, except PMs. Median intake: 37.4 g/day.
[b] Ham, bacon, sausage, and luncheon meats. Median intake: 14.8 g/day.
[c] No. of cases/no. of controls.
[d] ORs (and 95% CIs) adjusted by unconditional logistic regression for age, sex, ethnicity, pack-years of cigarette smoking, lifetime recreational physical activity (hours), lifetime aspirin use (months), body mass index 5 years ago, years of schooling, and intakes of nonstarch polysaccharides from vegetables and calcium from foods and supplements.
[e] *P* for interaction based on the likelihood ratio test comparing the model with interaction with one containing only main effects for the two variables (test has 1 degree of freedom).

CYP2E1 polymorphisms and intakes of salted/dried fish and Oriental pickled vegetables, foods that are other possible sources of nitrite and nitrosamines in our population. As for PM in Table 2, we found no strong association with colon cancer but a 1.5–3-fold increase in risk for rectal cancer among individuals with a high *CYP2E1* activity allele who were in the high consumption category for either food. However, the interaction tests were not statistically significant.

Because fruits and vegetables are rich in antioxidants, which may inhibit the nitrosation reaction, we reran the models for PM and colon and rectal cancer in Table 2, contrasting individuals who reported either a fruit and vegetable intake > median *and* a PM intake ≤ median (the low-risk category) and individuals who reported a fruit and vegetable intake ≤ median *and* a PM intake > median (the high-risk category; Table 4). Similar but somewhat stronger risk patterns were obtained. ORs were strongest for subjects who carried the high activity allele for either polymorphism and were in the high-risk intake category. The interaction tests did not reach statistical significance.

Discussion

In this population-based case-control study, we found that individuals carrying alleles that confer high CYP2E1 activity were at increased risk for rectal cancer. Individuals with the 5′ insert variant were at a statistically significant 60% increased risk of rectal cancer. Moreover, subjects who carry the insert and who were predicted to have been exposed to increased levels of NOCs, based on their high intake of RM or PMs, were at a markedly greater increased risk (2–3-fold) for rectal cancer. A very similar risk pattern was found for *CYP2E1* insert carriers who consumed salted/dried fish or Oriental pickled vegetables, two other foods that are sources of NOCs in our population. Finally, the combined association with PM and the *CYP2E1* insert appeared stronger among individuals with a low fruit and vegetable intake.

Dietary studies suggest that a high RM and PM intake increases CRC risk, whereas intake of white meat and (unprocessed) fish does not (1, 2). The associations with RM and PMs may result from the exposure of genetically susceptible individuals to chemical carcinogens. For example, we have previously reported in the same study that preference for well-done RM was associated with an 8.8-fold increased risk of CRC among ever-smokers with both the rapid NAT2 and rapid CYP1A2 phenotypes (who are better able to bioactivate HAAs), compared with ever-smokers with low NAT2 and CYP1A2 activities who preferred their RM rare or medium

Table 3 Joint effects of *CYP2E1* and salted/dried fish and oriental pickled vegetable intakes on CRC risk

Subsite	*CYP2E1*		Salted/dried fish (g/day)[a]				P^e	Oriental pickled vegetables (g/day)[b]				P
			0		>0			≤Median		>Median		
			n^c	OR[d] (95% CI)	n	OR (95% CI)		n	OR (95% CI)	n	OR (95% CI)	
Colon	*Rsa*I	*c1/c1*	228/386	1.0	43/63	1.1 (0.7–1.8)		105/229	1.0	166/220	1.7 (1.2–2.4)	
		Any *c2*	79/166	0.8 (0.6–1.1)	18/24	1.6 (0.8–3.0)	0.21	41/95	1.0 (0.6–1.6)	56/95	1.4 (0.9–2.1)	0.48
	Insert	*0*	217/402	1.0	44/66	1.3 (0.8–2.0)		100/237	1.0	161/231	1.7 (1.2–2.3)	
		1 or *2*	84/148	1.0 (0.7–1.5)	16/21	1.5 (0.7–2.0)	0.88	42/87	1.1 (0.7–1.7)	58/82	1.7 (1.1–2.7)	0.94
Rectum	*Rsa*I	*c1/c1*	96/386	1.0	24/63	1.5 (0.9–2.6)		42/229	1.0	78/220	1.9 (1.2–3.0)	
		Any *c2*	38/166	0.9 (0.6–1.4)	7/24	1.1 (0.4–2.9)	0.74	15/95	0.9 (0.5–1.8)	30/95	1.6 (0.9–2.9)	0.88
	Insert	*0*	87/402	1.0	17/66	1.1 (0.6–2.0)		38/237	1.0	66/231	1.7 (1.1–2.7)	
		1 or *2*	45/148	1.5 (1.0–2.4)	13/21	3.0 (1.4–6.6)	0.26	18/87	1.4 (0.7–2.6)	40/82	3.2 (1.8–5.7)	0.40

[a] Salted and dried fish, including taegu.
[b] Includes tsukemono, kim chee, and other Oriental pickled vegetables. Median intake: 1.64 g/day.
[c] No. of cases/no. of controls.
[d] ORs (and 95% CIs) adjusted by unconditional logistic regression for age, sex, ethnicity, pack-years of cigarette smoking, lifetime recreational physical activity (hours), lifetime aspirin use (months), body mass index 5 years ago, years of schooling, and intakes of nonstarch polysaccharides from vegetables and calcium from foods and supplements.
[e] P for interaction based on the likelihood ratio test comparing the model with interaction with one containing only main effects for the two variables (test has 1 degree of freedom).

Table 4 Joint effects of *CYP2E1*, PM, and fruit and vegetable intake on CRC risk

Subsite	*CYP2E1*		Intakes (g/day) of fruits and vegetables and PMs[a]				P^d
			High F&V, low PM		Low F&V, high PM		
			n^b	OR[c] (95% CI)	n	OR (95% CI)	
Colon	*Rsa*I	*c1/c1*	35/114	1.0	86/118	2.3 (1.4–3.9)	
		Any *c2*	20/49	1.5 (0.8–3.1)	20/45	1.4 (0.7–2.9)	0.05
	Insert	*0*	38/117	1.0	80/123	1.9 (1.2–3.1)	
		1 or *2*	16/44	1.3 (0.6–2.6)	26/40	2.1 (1.0–4.0)	0.69
Rectum	*Rsa*I	*c1/c1*	19/114	1.0	46/118	2.3 (1.2–4.4)	
		Any *c2*	7/49	1.0 (0.4–2.9)	14/45	1.7 (0.7–3.9)	0.60
	Insert	*0*	18/117	1.0	32/123	1.7 (0.8–3.3)	
		1 or *2*	8/44	1.6 (0.6–4.3)	28/40	5.0 (2.2–11.4)	0.27

[a] High F&V, low PM, fruit and vegetable intake > median (684 g/day) and PM intake ≤ median (14.8 g/day). Low F&V, high PM, fruit and vegetables intake ≤ median and processed meat intake > median.
[b] No. of cases/no. of controls.
[c] ORs (and 95% CIs) adjusted by unconditional logistic regression for age, sex, ethnicity, pack-years of cigarette smoking, lifetime recreational physical activity (hours), lifetime aspirin use (months), body mass index 5 years ago, years of schooling, and intake of calcium from foods and supplements.
[d] P for interaction based on the likelihood ratio test comparing the model with interaction with one containing only main effects for the two variables (test has 1 degree of freedom).

(11). Meat cooked well done is likely to contain HAAs and PAHs that are known carcinogens in animals (3).

The increased CRC risk with meat consumption could also result from the nitrosation reaction of amines with nitrite to form NOCs in the digestive tract. NOCs from Oriental pickled vegetables or salted/dried fish are thought to increase risk of stomach and nasopharyngeal cancer, respectively (1). In the colon, nitrite would be provided from PMs or by reduction of nitrate by colonic bacteria. The use of nitrite in cured and smoked meat was common in the past but has decreased substantially starting in the early 1960s (1, 4). Nitrosamines are now found in levels <10 ppb in cured meats (4). However, in the colonic lumen, amides and amines produced by bacterial degradation of amino acids can be *N*-nitrosated in the presence of nitrite (15). The latter may be formed by the reduction of nitrate from foods and water by the colonic flora (16). High-meat diets have been shown to increase fecal concentration of NOCs. Suzuki and Mitsuoka (5) found that volatile nitrosamines markedly increase in the feces of Japanese fed a Western diet rich in bacon and beef for 8 days. Similarly, Bingham *et al.* (6) recently showed in eight men kept for 3 weeks in a metabolic ward that fecal levels of NOCs increased 4-fold on a high-RM diet (600 g/day) compared with a low-RM diet (60 g/day) or a high-white meat and fish diet with similar caloric and fat contents. Fecal nitrite was also shown to increase after changing from a white meat to RM diet. Thus, there is mounting evidence that nitrosamine exposure to the large bowel increases on a high-RM and -PM diet.

Endogenous formation of NOCs can also occur by reaction of amines with products of nitric oxide (NO) generated during inflammation and infection (4). Activated macrophages produce NO from arginine by the inducible NO synthase pathway (4). Increased levels of arginine from a high-protein diet have been shown in rats to increase urine excretion of endogenously produced nitrate (17). Thus, under conditions of chronic inflammation or infection, and perhaps particularly on a high-protein diet, substantial amounts of NO are produced that react with oxygen to form nitrosating agents. Chronic inflammation, such as in ulcerative colitis, is thought to increase CRC risk (1).

Besides diet, exogenous exposure to nitrosamines can occur through use of tobacco products, cosmetics, pharmaceutical products, and agricultural chemicals and in certain occupational

settings (4). Smoking has been associated with CRC in a number of recent studies (1).

Nitrosamines are potent carcinogens in several animal models and are characterized by an organ selectivity that varies across species (4). Although large bowel tumors have not been commonly observed in experimental animals treated with nitrosamines, a different organ specificity that would include the rectum is possible in humans. CYP2E1 is the major enzyme involved in the α-hydroxylation of low molecular weight nitrosamines to yield compounds able to react with DNA at a number of different sites in a manner typical of alkylating agents. Some of the resulting adducts (*e.g.*, O^6-methylguanine) are mutagenic and cause GC→AT transition mutations (18). Such adducts have been detected in human colonic tissue (19), and *N*-methyl-*N*-nitrosourea has been shown to induce G→A transitions in codons 12 and 13 of K-*ras* in 30% of induced tumors in the rat colon (20). These mutations are commonly found in human colorectal tumors.

Considerable interindividual variability in human CYP2E1 activity has been demonstrated both *in vitro* using liver microsomes (21, 22) and *in vivo* based on the 6-hydroxylation of chlorzoxazone as a probe (23, 24). Pathophysiological factors, such as increased body weight, prolonged fasting, and liver dysfunction, and exogenous factors, such as ethanol, isothiocyanates, and certain medications (*e.g.*, acetaminophen, disulfiram, chlormethiazole), are known to modulate CYP2E1 activity (reviewed in Ref. 8). In addition, inherited genetic alterations contribute to its variance. A polymorphism in the 5′-flanking region of the *CYP2E1* gene (G1259C), detected with the restriction enzyme *Rsa*I or *Pst*I, has been reported with a frequency of 2–8% in whites and African Americans and 24% in Japanese. It has been shown to confer a reduced enzyme activity or inducibility (8, 9) and had been associated with a reduced risk of lung and esophageal cancers (25, 26). A second (unlinked) functional polymorphism has recently been described in the regulatory region of the gene and consists of a 96-bp insertion observed with a frequency of 3% in Caucasians (10), 15% in African Americans (10), and 23% in Japanese (this study). It has been associated with an enhanced CYP2E1 metabolic ability in the presence of ethanol intake or obesity (10), two factors known to increase CRC risk.

This is the first study to examine the association of these polymorphisms with CRC. Individuals with the high-activity alleles were found to be at increased risk for rectal cancer, although this association was stronger for the insert polymorphism than for *Rsa*I. Interestingly, for both RM and PM, the highest rectal cancer risk was found in subjects who carried the insert and had a high intake of these foods. Similar risk patterns were found for other foods, namely salted/dried fish and Oriental pickled vegetables, which are known sources of nitrite and nitrosamines and are risk factors for other cancers (1, 27). Also consistent with a role for nitrosamines is the stronger effect found in subjects with a low intake of fruits and vegetables, foods rich in antioxidants that may inhibit the nitrosation reaction. Although the tests for interaction were not statistically significant, the data suggest that subjects with the insert and a high intake of RM and PM may be at increased risk of rectal cancer (possibly because of their better ability to activate nitrosamines) and that RM and PMs are significant sources of exposure to precursors for these compounds. Thus, in humans, the rectum may be a target organ for the carcinogenicity of nitrosamines. This is consistent with the increased rectal cancer risk reported in beer drinkers in cohort studies conducted several decades ago, when beer may still have contained significant amounts of nitrosamines (28, 29).

The response rate was less than optimal in this study. However, we found little indication that nonparticipants differ in any substantial way from the interviewed subjects (see "Materials and Methods"). Similarly, refusal to provide a blood sample was not associated with lifestyle factors in this study (30). It seems unlikely that selection or recall bias could explain the observed associations because any bias would have had to operate preferentially for rectal cancer.

In summary, these data provide additional support for the hypothesis that nitrosamines are carcinogenic to the rectum in humans and that RM and, in particular, PMs are significant sources of exposure for these compounds.

Acknowledgments

We thank the Hawaii Tumor Registry, Castle Medical Center, Kaiser-Permanente Medical Center, Kuakini Medical Center, Queen's Medical Center, Straub Clinic and Hospital, St. Francis Medical Center, and Wahiawa General Hospital for collaboration. We also thank Aleli Vinoya for assistance with data analysis.

References

1. World Cancer Research Fund/American Institute for Cancer Research. Food Nutrition and the Prevention of Cancer: A Global Perspective. Washington, DC: American Institute for Cancer Research, 1997.

2. Sandhu, M. S., White, I. R., and McPherson, K. Systematic review of the prospective cohort studies on meat consumption and colorectal cancer risk: a meta-analytical approach. Cancer Epidemiol. Biomark Prev., *10:* 439–446, 2001.

3. Sugimura, T. Carcinogenicity of mutagenic heterocyclic amines formed during the cooking process. Mutat. Res., *150:* 33–41, 1985.

4. Hecht, S. S. Approaches to cancer prevention based on an understanding of *N*-nitrosamine carcinogenesis. Proc. Soc. Exp. Biol. Med., *216:* 181–191, 1997.

5. Susuki, K., and Mitsuoka, T. Increase in faecal nitrosamines in Japanese individuals given a Western diet. Nature (Lond.), *294:* 453–456, 1981.

6. Bingham, S. A., Pignatelli, B., Pollock, J. R. A., Ellul, A., Malaveille, C., Gross, G., Runswick, S., Cummings, J. H., and O'Neill, I. K. O. Does increased endogenous formation of *N*-nitroso compounds in the human colon explain the association between red meat and colon cancer? Carcinogenesis (Lond.), *17:* 515–523, 1996.

7. Yang, C. S., Yoo, J. S. H., Ishizaki, H., and Hong, J. Cytochrome P450IIE1: role in nitrosamine metabolism and mechanisms of regulation. Drug Metab. Dispos., *22:* 147–159, 1990.

8. Le Marchand, L., Wilkinson, G. R., and Wilkens, L. R. Genetic and dietary predictors of CYP2E1 activity: a phenotyping study in Hawaii Japanese using chlorzoxazone. Cancer Epidemiol. Biomark. Prev., *8:* 495–500, 1999.

9. Lucas, D., Menez, C., Girre, C., Berthou, F., Bodenez, P., Joannet, I., Hispard, E., Bardou, L-G., and Menez, J-F. Cytochrome *P4502E1* genotype and chlorzoxazone metabolism in healthy and alcoholic Caucasian subjects. Pharmacogenetics, *5:* 298–304, 1995.

10. MacCarver, D. G., Byun, R., Hines, R. N., Hichme, M., and Wegenek, W. A genetic polymorphism in the regulatory sequences of human *CYP2E1*: association with increased chlorzoxazone hydroxylation in the presence of obesity and ethanol intake. Toxicol. Appl. Pharmacol., *152:* 276–281, 1998.

11. Le Marchand, L., Hankin, J. H., Wilkens, L. R., Pierce, L. M., Franke, A. A., Kolonel, L. N., Seifried, A., Custer, L. J., Chang, W., and Lum-Jones, A. Combined effect of well-done red meat, smoking and rapid NAT2 and CYP1A2 phenotypes in increasing colorectal cancer risk. Cancer Epidemiol. Biomark. Prev., *10:* 1259–1266, 2001.

12. Hankin, J. H., Wilkens, L. R., Kolonel, L. N., and Yoshizawa, C. N. Validation of a quantitative diet history method in Hawaii. Am. J. Epidemiol., *133:* 616–628, 1991.

13. Breslow, N. E., and Day, N. E. Statistical Methods in Cancer Research, Vol 1. The Analysis of Case-Control Studies. IARC Scientific Pub. No. 32. Lyon, France: IARC, 1980.

14. Willett, W. C., and Stampfer, M. J. Total energy intake: implications for epidemiologic analyses. Am. J. Epidemiol., *124:* 17–27, 1986.

15. Bingham, S. A. Meat or wheat for the next millenium? Plenary lecture: high-meat diets and cancer risk. Proc. Nutr. Soc., *58:* 243–248, 1999.

16. Allison, C., and Macfarlane, G. T. Effect of nitrate on methane production and fermentation in slurries of human faecal bacteria. J. Gen. Microbiol., *134:* 1397–1405, 1988.

17. Mallett, A. K., Walters, D. G., and Rowland, I. R. Protein related differences in the excretion of nitrosoproline and nitrate by the rat. Food Chem. Toxicol., *26:* 831–835, 1988.

18. Loveless, A. Possible relevance of O^6 alkylation of deoxyguanosine to the mutagenicity and carcinogenicity of nitrosamines and nitrosamides. Nature (Lond.), *233:* 206–207, 1969.

19. Hall, C. N., Badawi, A. F., O'Connor, P. L., and Saffhill, R. The detection of DNA damage in the DNA of human gastrointestinal tissue. Br. J. Cancer, *64:* 59–63, 1991.

20. Jacoby, R. F., Alexander, R. J., Raicht, R. F., and Brasitus, T. A. K-*ras* oncogene mutations in rat colon tumors induced by *N*-methyl-*N*-nitrosourea. Carcinogenesis (Lond.), *13:* 45–49, 1992.

21. Yoo, J. S. H., Guengerich, F. P., and Yang, C. S. Metabolism of *N*-nitrosodialkylamines by human liver microsomes. Cancer Res., *48:* 1499–1504, 1988.

22. Hunt, C. M., Strater, S., and Stave, E. M. Effects of normal aging on the activity of human hepatic cytochrome P450IIE1. Biochem. Pharmacol., *10:* 1666–1669, 1990.

23. Kim, R. B., O'Shea, D., and Wilkinson, G. R. Interindividual variability of chlorzoxazone 6-hydroxylation in men and women and its relationship to CYP2E1 genetic polymorphisms. Clin. Pharmacol. Ther., *57:* 645–655, 1995.

24. Girre, C., Lucas, S. N., Hispard, E., Menez, C., Dally, S., and Menez, J-F. Assessment of cytochrome P450IIE1 induction in alcoholic patients by chlorzoxazone pharmacokinetics. Biochem. Pharmacol., *47:* 1504–1508, 1994.

25. Le Marchand, L., Sivaraman, L., Pierce, L., Seifried, A., Lum, A., Wilkens, L. R., and Lau, A. F. Associations of *CYP1A1*, *GSTM1*, and *CYP2E1* polymorphisms with lung cancer suggest cell type specificities to tobacco carcinogen. Cancer Res., *58:* 4858–4863, 1998.

26. Tan, W., Song, N., Wang, G. Q., Liu, Q., Tang, H. J., Kadlubar, F. F., and Lin, D. X. Impact of genetic polymorphisms in cytochrome P4502E1 and glutathione *S*-transferase M1, T1, and P1 on susceptibility to esophageal cancer among high-risk individuals in China. Cancer Epidemiol. Biomark. Prev., *9:* 551–556, 2000.

27. Haorah, J., Zhou, L., Wang, X., Xu, G., and Mirvish, S. S. Determination of total *N*-nitroso compounds and their precursors in frankfurters, fresh meat, dried salted fish, sauces, tobacco, and tobacco smoke particulates. J. Agric. Food Chem., *49:* 6068–6078, 2001.

28. Dean, G., MacLennan, R., McLoughlin, H., and Shelley, E. Causes of death of blue-collar workers at a Dublin brewery. Br. J. Cancer, *40:* 581–589, 1979.

29. Jensen, O. M. Cancer morbidity and causes of death among Danish brewery workers. Int. J. Cancer, *23:* 454–463, 1979.

30. Le Marchand, L., Donlon, T., Hankin, J. H., Kolonel, L. N., Wilkens, L. R., and Seifried, A. B-vitamin intake, metabolic genes and colorectal cancer risk. Cancer Causes Control, *13:* 239–248, 2002.

Red Meat Intake, *CYP2E1* Genetic Polymorphisms, and Colorectal Cancer Risk

Loïc Le Marchand, Timothy Donlon, Ann Seifried, et al.

Cancer Epidemiol Biomarkers Prev 2002;11:1019-1024.