Associations between Smoking, Passive Smoking, *GSTM-1*, *NAT2*, and Rectal Cancer[1]

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Abstract

Cigarette smoking has been identified as a risk factor for colon cancer, however, much less is known about the association between cigarette smoking and rectal cancer. The purpose of this article is to evaluate the associations between rectal cancer and active and passive cigarette smoking and other forms of tobacco use. We also evaluate how genetic variants of *GSTM-1* and *NAT2* alter these associations. A population-based case-control study of 952 incident rectal cancer cases and 1205 controls was conducted. Detailed tobacco use information was collected as part of an interviewer-administered questionnaire. DNA was extracted from blood to examine genetic variants of *GSTM-1* and *NAT2*. Cigarette smoking was associated with an increased risk of rectal cancer in men [odds ratio (OR) = 1.5, 95% confidence interval (CI), 1.1–2.1 for current smokers; OR = 1.7, 95% CI, 1.3–2.3 for smoking >20 pack-years of cigarettes relative to never-smokers]. After adjusting for active smoking, exposure to cigarette smoke of others also was associated with increased risk among men (OR = 1.5, 95% CI, 1.1–2.0). Neither *GSTM-1* genotype nor NAT2-imputed phenotype was independently associated with rectal cancer. However, the risk associated with smoking cigarettes among those who were *GSTM-1* null relative to those who never smoked and had the *GSTM-1* present genotype was OR = 2.0 (95% CI, 1.2–3.3). This interaction was of borderline significance ($P = 0.08$). Men who had the combined *GSTM-1* present genotype and who were rapid acetylators had no increased risk from cigarette smoking. There were no significant associations between cigarette smoking and rectal cancer among women. This study shows that men who smoke cigarettes, especially those who smoke >20 pack-years, are at increased risk of rectal cancer. This association may be influenced by *GSTM-1* genotype. Furthermore, exposure to cigarette smoke of others may increase risk of rectal cancer among men who do not smoke.

Introduction

Colon cancer has been added to the list of cancers associated with cigarette smoking (1). Both usual number of cigarettes smoked/day (2) and pack-years smoked (3) have been identified as indicators of risk, with identical risk estimates being reported from large case-control and cohort studies (2, 3). The level of risk is generally reported as an increase of 40–50% at the population level, perhaps making it difficult for small studies to detect associations. Although, many other robust studies have since verified these associations with cigarette smoking, the findings are not universal (4). Cigarette smoking has been linked to specific types of tumor alterations, lending additional support for an association between cigarette smoking and colon cancer (5, 6).

The observed associations between smoking cigarettes and increased risk of colon cancer have led to other questions. First, are the associations between cigarette smoking and rectal cancer the same as those observed for colon cancer? Studies of colorectal cancer have been done, although most of the cases are in the colon rather than rectum, with power to detect associations for rectal cancer being limited. Secondly, is passive smoking associated with colorectal cancer? The association between passive smoking and colorectal cancer has not been examined.

The association between cigarette smoking and colorectal cancer might be modified by the ability to detoxify polycyclic aromatic hydrocarbons generated from smoking cigarettes. To this end, genetic variants of Phase II-detoxifying enzymes such as *GST* and *NAT2* have been examined in conjunction with cigarette smoking. Associations between colon cancer and genetic variants of *GSTM-1* and *NAT2* are inconsistent. Larger studies generally show minimal interaction, whereas smaller studies often suggest an interaction (7–9).

In this study, we examine the association between cigarette smoking, passive smoking, *GSTM-1* genotype, and *NAT2*-imputed phenotype with risk of developing rectal cancer. We further evaluate how genetic variants of *GSTM-1* and *NAT2* may alter susceptibility to both active and passive smoking. We evaluate these associations in men and women and by age at time of diagnosis.

Materials and Methods

Study Population. Participants in the study were from the Kaiser Permanente Medical Care Program of Northern California and the state of Utah. All eligible cases within these defined geographic areas were identified and recruited for the study. Cases with a first primary tumor in the rectosigmoid junction or

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rectum were identified between May 1997 and May 2001. Case eligibility was determined by the Surveillance Epidemiology and End Results Cancer Registries in Northern California and Utah. Cases were identified using rapid-reporting systems. To be eligible, cases could not have had a previous colorectal tumor, had to be between 30 and 79 years of age at diagnosis, English speaking, and mentally competent to complete the interview. Cases with known (as indicated on the pathology report) familial adenomatous polyposis, ulcerative colitis, or Crohn's disease were not eligible.

Controls were matched to cases by sex and by 5-year age groups, using the same eligibility criteria as was used for cases. At the Kaiser Permanent Medical Care Program, controls were randomly selected from membership lists, and in Utah, controls ≥65 years were randomly selected from Health Care Financing Administration lists; controls < 65 years in Utah were randomly selected from driver's license lists.

A total of 982 rectal cancer cases and 1231 controls was interviewed between October 1997 and January 2002. The study response rate was 65.2% for cases and 65.3% for controls, representing the percentage interviewed based on those who were identified; the cooperation rate was 73.2% for cases and 68.8% for controls taking into account the number interviewed of those whom we were able to contact. Of these interviewed cases and controls, 56 were excluded from the analysis because they reported Crohn's disease or ulcerative colitis at the interview, had missing data, or data were considered to be of poor quality by the interviewer. A total of 952 rectal cancer cases and 1205 matched controls are included in the analyses presented. The race/ethnicity of the study population reported at the time of interview was 82% white, non-Hispanic, 4.1% African American, 7.6% Hispanic, 4.6% Asian, 0.7% Native American, and 1% multiple races/ethnicity.

Data Collection. Data were collected by trained and certified interviewers using laptop computers. Data for the rectal cancer study were collected using the same study questionnaire and the same quality control procedures as were used in our previous colon cancer study (10). Study participants were asked to recall the year 2 years before the date of selection (the date of diagnosis for cases or date of selection for controls). The interview took 2–3 h.

Cigarette Smoking History. Information was obtained about use of cigarettes, cigars, and pipes. We collected Information about ever using these tobacco products on a regular basis defined as at least 100 cigarettes during your lifetime and regular use of cigars or pipes defined as having used these products for at least 1 year. For each type of tobacco use reported, we obtained information on age started using the tobacco product, age stopped using the tobacco products, and usual number of cigarettes, cigars, or pipes smoked/day. Because some people do not smoke continuously, we also asked total number of years smoked. Pack-years of cigarettes smoked was determined by multiplying the usual number of cigarettes smoked/day times total years of smoking cigarettes and dividing by 20 or a pack of cigarettes. Exposure to ETS[3] was obtained by asking usual number of hours/week exposed to cigarette smoke in the house and out of the house for the referent period and 10 and 20 years ago.

Other Information. Other information obtained included information on moderate and vigorous physical activity performed over the past 20 years, reported height and weight 2 years ago before selection/diagnosis, long-term alcohol use as well as more detailed alcohol consumption during the referent year, family history of cancer, use of aspirin and nonsteroidal anti-inflammatory drugs, reproductive history, and detailed dietary intake information using an adaptation of the CARDIA diet history that was modified for use in case-control studies to be administered on a laptop computer (11).

Genetic Data. Blood was drawn on study participants, and DNA was extracted. The *GSTM-1* null genotype was detected using the PCR method described by Zhong *et al.* (12). Briefly, the PCR reaction uses three primers. The P3 primer sequence is specific for the *GSTM-1* gene and when used with P1 results in a 230-bp product specific for *GSTM-1*. The P1-P2 primer pair is nonspecific for *GSTM-1* or *GSTM-4* and results in a 157-bp product. The PCR products are run on a 2% agarose gel and stained with ethidium bromide. If the only product displayed is the 157-bp product, it is classified as *GSTM-1* null, whereas if both bands are present, it is classified as *GSTM-1* positive.

Three variants of NAT2 were assessed, using the method of Bell *et al.* (13), to determine acetylator status. These three variants account for ~90–95% of the slow acetylation phenotype in Caucasians; because a small percentage of the population was African American, we did not obtain information on the G191A variant that is more common in African-American populations. All three variants we assessed could be identified from one PCR product and be digested with three different restriction enzymes. The C481T variants were determined using a *Kpn*I digest, the G590A variant using a *Taq*I digest, and the G857A variant using a *Bam*HI digest. Those with at least 2 variant alleles were classified as slow acetylators. Those with 1 or 0 variant alleles were classified as fast acetylators.

Statistical Analysis. The distribution of cigarette, cigar, and pipe smoking in the population was assessed for men and women separately. We evaluated pack-years of cigarettes smoked, usual number of cigarettes smoked a day, age started to smoke cigarettes, and years since having stopped smoking cigarettes. Passive smoking was evaluated by the total number of hours exposed to others smoke in the home, away from the home, and a combination of both in and away from the home. Cutpoints for smoking were based on distribution in the controls, except for evaluation of usual amount smoked/day, where the pack a day or 20 cigarette cutpoint was used.

Associations were evaluated using unconditional multiple logistic regression models adjusting for age at diagnosis or selection, body size, physical activity patterns, and alcohol intake. Covariates evaluated in the model were those that could be associated with both smoking and with rectal cancer. In models that were used to evaluate associations with passive smoking, adjustment for cigarette smoking status also was done. This was done because smokers also may have more passive smoke exposure, and we wanted to separate out the effects of passive smoking from those for smoking. We also evaluated associations by disease stage because cigarette smokers may stop because of illness; if associations were stronger for those with distant disease stage, it is possible that associations were underestimated. ORs and 95% CIs are reported. Interaction between smoking and genotype was assessed by including a cross-product term in the model along with the independent smoking and genotype variables. The improvement in the fit of the logistic model with the interaction term was determined using the χ^2 statistic with one degree of freedom.

[3] The abbreviations used are: ETS, environmental tobacco smoke; OR, odds ratio; CI, confidence interval; AJCC, American Joint Committee on Cancer; MSI, microsatellite instability; CARDIA, coronary artery risk development in young adults.

Table 1 Description of study population

	Men		Women	
	Cases *n* %	Controls *n* %	Cases *n* %	Controls *n* %
Age (yr)				
<45	36 (6.4)	43 (6.4)	37 (9.4)	42 (7.9)
45–54	112 (20.0)	134 (19.9)	85 (21.6)	106 (19.9)
55–64	176 (31.5)	193 (28.7)	114 (29.0)	156 (29.3)
65–74	162 (29.0)	206 (30.6)	103 (26.2)	139 (26.1)
75–79	73 (13.1)	97 (14.4)	54 (13.7)	89 (16.7)
Education level				
<High school	57 (10.2)	76 (11.3)	47 (12.0)	51 (9.6)
High school	125 (22.4)	126 (18.8)	101 (25.7)	144 (27.1)
Some college	278 (49.7)	323 (48.1)	195 (49.6)	255 (47.9)
College graduate +	99 (17.7)	147 (21.9)	50 (12.7)	82 (15.4)
Cigarette smoker				
Never	209 (37.6)	297 (44.2)	232 (59.2)	328 (61.7)
Stopped ≥5 yr	229 (41.2)	272 (40.5)	93 (23.7)	118 (22.2)
Current (<5 yr)	118 (21.2)	103 (15.3)	67 (17.1)	86 (16.2)
Cigar smoker				
Never	505 (90.3)	609 (90.6)	392 (99.8)	530 (99.6)
Ever	54 (9.7)	64 (9.4)	1 (0.2)	2 (0.4)
Pipe smoker				
Never	499 (89.3)	598 (88.9)	393 (100)	531 (99.8)
Ever	60 (10.7)	75 (11.1)	0	1 (0.2)

Results

The majority of cases were between 45 and 75 years of age (Table 1). Controls reported slightly more education than cases. Approximately 40% of men and 60% of women reported never smoking cigarettes on a regular basis. Of those who ever smoked cigarettes, the majority had stopped smoking >5 years before diagnosis or selection. There were few men who smoked cigars (~10%) and pipes (~11%). There was a significant difference in both men and women (χ^2, $P < 0.001$) in the proportion of current smokers within 5 years before diagnosis and never or ex-smokers who were exposed to long-term passive smoke. Among smokers, 74% of men and 60% of women were in the highest category of passive smoking, whereas 42% of men and 32% of women who were never or ex-smokers reported being in the high passive smoking category.

Current cigarette smoking was associated with a 50% increased risk of rectal cancer in men (Table 2). There was no trend between age starting to smoke and risk of rectal cancer. People who reported having stopped smoking cigarettes >5 years before diagnosis or selection were not at increased risk of rectal cancer. Slightly stronger associations were observed for pack-years having smoked than for usual number of cigarettes smoked. Among women, we did not observe a significant increased risk of rectal cancer associated with smoking cigarettes; stratification by estrogen status also did not yield significant associations for women who were estrogen positive or estrogen negative. However, among estrogen-positive women, the point estimate was similar to that observed for men, although CIs were wide (OR = 1.4, 95% CI, 0.8–2.3). Neither cigar nor pipe smoking was associated with increased risk among men; too few women smoked these tobacco products to reliably estimate risk. Adjustment for sigmoidoscopy screening did not alter findings.

Evaluation of ETS both inside and outside of the home showed that ETS was significantly associated with rectal cancer in men but not women after adjusting for cigarette smoking practices (Table 3). The strength of the association was similar to that observed for active smoking. Stratification by current *versus* not-current smokers showed that the stronger associations were among nonsmokers (OR = 1.5, 95% CI, 1.1–2.2), however, very few smokers were not exposed to passive smoking (8 cases and 4 controls), making any estimate of association very imprecise (95% CI, 0.1–1.9). Strongest associations were observed for recent and long-term ETS in the home (OR = 1.5), and total ETS 10 and 20 years ago (OR = 1.5, 95% CI, 1.1–2.0).

Evaluation of associations between both active and passive smoking by age showed that associations were slightly stronger among older men (data not shown in table). The OR associated with being a current smoker for men >65 years was 2.0 (95% CI, 1.1–3.9) compared with an OR of 1.3 (95% CI, 0.9–2.0) for men ≤65 years of age. Likewise, pack-years of cigarettes smoked also were more strongly associated with rectal cancer risk among older men (OR = 2.0, 95% CI, 1.3–3.2 for men > 65 years; OR = 1.5, 95% CI, 1.0–2.1 for men ≤65 years). This may reflect greater number of pack-years smoked by older men (mean for younger men 14.4 pack-years and for older men 21.6 pack-years). Although associations were slightly stronger among women diagnosed when older, they were not statistically significant.

Evaluation of tobacco exposure by disease stage showed stronger associations for both men and women diagnosed at a distant (AJCC stage 4) disease stage (data not shown in table), although estimates of association were much less precise for AJCC stage 4. Among men, the association between being a current smoker relative to never having smoked was OR = 1.3 (95% CI, 0.9–1.9) for AJCC stages 1–3 and OR = 2.1 (95% CI, 0.9–4.9) for AJCC stage 4; the association for >20 pack-years relative to never having smoked was OR = 1.6 (95%CI, 1.2–2.2) for AJCC stages 1–3 and OR = 2.7 (95% CI, 1.2–6.0) for AJCC stage 4; and long-term exposure to passive smoking of >10 h/week relative to none was OR = 1.3 (95% CI, 0.9–1.9) for AJCC stages 1–3 and OR = 4.5 (95% CI, 1.3–15.8) for AJCC stage 4. Similar associations for women for being a current smoker was OR = 1.0 (95% CI, 0.6–1.5) for AJCC stages 1–3 and OR = 1.7 (95% CI, 0.5–5.6) for AJCC stage 4; for pack-years smoked was OR = 1.1 (95% CI, 0.7–1.6) for AJCC stages 1–3 and OR = 2.4 (95% CI, 0.8–7.5) for AJCC stage 4; and for passive smoke exposure was OR = 0.9 (95% CI, 0.6–1.3) for AJCC stages 1–3 and OR = 1.2 (95% CI, 0.4–4.3) for AJCC stage 4. These associations did not appear to be confounding by sigmoidoscopy screening.

Neither *GSTM-1* genotype nor NAT2-imputed phenotype was associated with rectal cancer in either men or women (Table 4). There was a slight suggestion of a decreased risk for the combined *GSTM-1* null genotype and NAT2-slow-imputed phenotype in women (OR = 0.6; 95% CI, 0.4–1.0) but not in men.

Assessment of the combined effects of cigarette smoking and genotype in men (Table 5) showed stronger associations with having smoked cigarettes within the past 5 years and also having the *GSTM-1* null genotype relative to never having smoked cigarettes and having the *GSTM-1* present genotype. This interaction was of borderline significance on the multiplicative scale of interaction ($P = 0.08$) and showed a 60% greater risk on the additive scale for the combined *GSTM-1* null genotype and being a current smoker than would be expected for the two exposures independently. Current smokers who were fast acetylators were at slightly lower risk than current smokers who were slow acetylators. The risk was slightly less than would be expected on an additive scale. Evaluation of the combined *GSTM-1* genotype and NAT2-imputed phenotype among men showed that current smokers who had a combined

Table 2 Associations between cigarette smoking and rectal cancer risk in men and women

	Men			Women		
	Cases (*n*)	Controls (*n*)	OR (95% CI)[a]	Cases (*n*)	Controls (*n*)	OR (95% CI)
Cigarette smoking						
Never	209	297	1.0	232	328	1.0
Former	229	272	1.2 (0.9–1.6)	93	118	1.0 (0.7–1.5)
Current (<5 yr)	118	103	1.5 (1.1–2.1)	67	86	1.0 (0.7–1.5)
Age started smoking						
Never	209	297	1.0	232	328	1.0
≤16 yr	153	166	1.2 (0.9–1.7)	50	56	1.1 (0.7–1.7)
17–20 yr	137	133	1.5 (1.1–2.0)	65	83	1.1 (0.7–1.6)
>20 yr	60	77	1.1 (0.8–1.7)	46	65	1.0 (0.6–1.5)
Years, Since stopping						
Never smoked	209	297	1.0	232	328	1.0
≥15 yrs	170	200	1.2 (0.9–1.6)	66	80	1.1 (0.7–1.6)
5–14 yrs	59	72	1.1 (0.8–1.7)	27	38	1.0 (0.6–1.7)
Current (<5 yr)	118	103	1.5 (1.1–2.1)	67	86	1.0 (0.7–1.5)
Pack-years[b]						
Never	211	298	1.0	233	329	1.0
≤20	132	194	1.0 (0.7–1.3)	73	108	0.9 (0.6–1.3)
>20	215	179	1.7 (1.3–2.3)	83	92	1.2 (0.8–1.7)
Usual number cigarettes/day						
Never smoked	209	298	1.0	232	328	1.0
<20	258	276	1.3 (1.01–1.7)	130	172	1.0 (0.7–1.4)
≥20	92	100	1.3 (0.9–1.8)	30	30	1.2 (0.7–2.2)

[a] Adjusted for age, physical activity level, alcohol intake, and body size.
[b] People who smoked <0.5 pack-years were classified as never having smoked, thus numbers across categories differ slightly.

GSTM-1 present genotype and were rapid acetylators were not at increased risk from smoking cigarettes; the other combinations of genotypes were at increased risk from cigarette smoking (Table 6).

Discussion

We detected similar associations between smoking cigarettes and rectal cancer for men as we have previously reported for colon cancer (2). Contrary to our previous observations between cigarette smoking and colon cancer for women, we did not observe an increased risk among women with either smoking more cigarettes/day or having smoked more pack-years. Although there was some suggestion of similar risk as observed for men among women who were estrogen positive, we did not have enough power to evaluate this with any precision. However, women smoked much less than men, with the highest group of smokers being estrogen-negative women who smoked 12.8 pack-years, which was much less than the 21.6 pack-years reported by older men. We also observed that exposure to cigarette smoke of others increased risk of colon cancer after adjusting for active smoking among men. This is one of the first studies to examine the effects of ETS or rectal cancer risk.

The understanding of the association between cigarette smoking and colorectal cancer has emerged through many, somewhat seemingly contradictory observations (14). For many years, it was noted that cigarette smoking was associated with adenomas but not with carcinomas (4). However, with observations from some large colon cancer studies and cohorts with adequate numbers of cases, a modest association—roughly a 40–50% increase in risk—between cigarette smoking and colon cancer was observed. It is possible that given the modest level of association that small case-control and cohort studies with few cases did not have adequate power to detect associations. It also has been proposed that early studies examined cigarette smoking in too crude of a manner to see an association (15), although many studies that used similar indicators of cigarette smoking as we presented in this article, did not see associations (16). Credibility to the association between smoking cigarettes and colon cancer has been gained from additional studies reporting associations and from observations that cigarette smoking is associated with specific types of alterations in tumors (5, 6). These data suggest similar associations with rectal cancer, at least among men.

Data are mixed in the associations between colon and rectal cancer. Some studies report stronger associations with cigarette smoking for rectal tumors than for colon tumors (17–19), others report similar associations for both colon and rectal tumors (20, 21), and some studies do not observe significant associations between cigarette smoking and either colon or rectal cancer (22–25). Some of these studies have been restricted to women (18, 19, 22), whereas others have been restricted to men (25), and most have few cases of rectal cancer. The strongest association reported was between heavy smokers of a pack or more a day where the risk for rectal cancer was over 5, although for colon cancer it was a nonsignificant 1.7 (19). The study by Ji *et al.* (20) reported a nonsignificant risk of 1.5 for rectal cancer among those who smoked ≥55 pack-years. LeMarchand *et al.* (21) reported stronger associations for distal colon tumors than for either proximal or rectal tumors. They reported a nonsignificant risk of 1.3 for men and 1.5 for women for 39 and 28 pack-years, respectively. However, the study was limited to 221 cases of rectal cancer in men and 129 cases of rectal cancer in women, thus they had limited power to detect significant associations. Other forms of tobacco, such as cigar and pipe smoking also have been evaluated with rectal cancer. An increased risk of rectal cancer has been reported with cigar use (26); we did not detect significant associations with either cigar or pipe use among men in this study.

In our previous report on the association between cigarette smoking and colon cancer, we observed stronger associations

Table 3 Associations between passive smoking and rectal cancer in men and women

	Men			Women		
	Cases (*n*)	Controls (*n*)	OR (95% CI)[a]	Cases (*n*)	Controls (*n*)	OR (95% CI)
Passive smoking in the home (h/wk)						
Recent						
None	493	614	1.0	337	462	1.0
1–10	17	20	1.0 (0.5–2.0)	17	29	0.7 (0.4–1.4)
>10	47	36	1.5 (1.0–2.4)	37	38	1.1 (0.7–1.9)
10 years ago						
None	463	583	1.0	300	418	1.0
1–10	31	35	1.1 (0.6–1.7)	28	42	0.8 (0.5–1.4)
>10	61	52	1.3 (0.9–2.0)	60	64	1.2 (0.8–1.8)
20 years ago						
None	407	525	1.0	258	369	1.0
1–10	42	45	1.2 (0.8–1.9)	40	61	0.9 (0.6–1.4)
>10	101	93	1.3 (0.9–1.8)	86	97	1.2 (0.8–1.7)
Long-term average						
None	389	501	1.0	235	342	1.0
1–10	85	102	1.0 (0.7–1.4)	78	95	1.1 (0.8–1.6)
>10	75	58	1.5 (1.0–2.2)	68	83	1.0 (0.7–1.6)
Passive smoking outside the home (h/w)						
Recent						
None	316	417	1.0	258	369	1.0
1–10	152	179	1.1 (0.9–1.5)	103	151	0.8 (0.6–1.1)
>10	77	60	1.5 (1.0–2.2)	27	30	1.0 (0.6–1.8)
10 years ago						
None	214	289	1.0	181	243	1.0
1–10	185	239	1.1 (0.8–1.4)	147	202	0.9 (0.7–1.2)
>10	143	128	1.4 (1.0–1.9)	51	69	0.9 (0.6–1.3)
20 years ago						
None	135	185	1.0	138	199	1.0
1–10	181	252	1.0 (0.7–1.3)	154	204	1.0 (0.8–1.4)
>10	225	215	1.4 (1.0–1.8)	89	114	1.0 (0.7–1.5)
Long-term average						
None	113	154	1.0	113	144	1.0
1–10	148	221	0.9 (0.6–1.2)	126	180	0.9 (0.6–1.2)
>10	273	270	1.3 (1.0–1.8)	138	180	0.9 (0.6–1.2)
Total passive smoke (h/wk)						
Recent						
None	303	398	1.0	242	316	1.0
1–10	142	167	1.2 (0.9–1.5)	95	137	0.8 (0.6–1.1)
>10	99	90	1.3 (0.9–1.8)	50	61	0.9 (0.6–1.4)
10 years ago						
None	194	237	1.0	160	221	1.0
1–10	175	228	1.1 (0.8–1.5)	130	179	0.9 (0.6–1.2)
>10	170	155	1.5 (1.1–2.0)	87	110	0.9 (0.6–1.3)
20 years ago						
None	115	165	1.0	119	171	1.0
1–10	165	239	1.0 (0.7–1.4)	124	170	1.0 (0.7–1.4)
>10	256	244	1.5 (1.1–2.0)	135	175	1.0 (0.7–1.4)
Long-term average						
None	93	135	1.0	92	122	1.0
1–10	315	393	1.2 (0.8–1.6)	197	284	0.8 (0.6–1.2)
>10	122	113	1.4 (1.0–2.1)	83	94	1.0 (0.6–1.5)
Long-term average (10 and 20 yr ago)						
None	95	140	1.0	99	133	1.0
1–10	232	311	1.1 (0.8–1.5)	160	231	0.8 (0.6–1.2)
>10	205	195	1.5 (1.04–2.1)	114	142	0.9 (0.6–1.4)

[a] Adjusted for age, physical activity level, alcohol in take, usual cigarettes smoked/day (passive smoking only), and body size.

with usual number of cigarettes smoked than for pack-years smoked; similar findings have been reported by others for colorectal cancer (27). We attributed that observation to the importance of dose of cigarette smoke. Pack-years of cigarettes smoked also is an indicator of dose and, depending on the age of the population, may have variability in its association with dose. Other studies have found pack-years to be the strongest indicator of risk for colon cancer (3), and in our current study, we observed stronger associations with pack-years smoked than usual number of cigarettes smoked/day. This could be the result of more accurate reporting of total years smoked for rectal cancer study than for the colon cancer study.

Table 4 Associations between *GSTM-1* and *NAT2*-imputed phenotype and rectal cancer in men and women

	Men			Women		
	Cases (*n*)	Controls (*n*)	OR (95% CI)[a]	Cases (*n*)	Controls (*n*)	OR (95% CI)
GSTM-1						
Present	230	279	1.0	167	188	1.0
Null	243	295	1.0 (0.8–1.3)	161	251	0.7 (0.6–1.0)
NAT2 (acetylator status)						
Slow	247	306	1.0	162	214	1.0
Rapid	204	255	1.0 (0.8–1.3)	153	215	0.9 (0.7–1.3)
GSTM-1/*NAT2* combined						
Present/slow	130	148	1.0	83	87	1.0
Present/rapid	89	125	0.8 (0.6–12)	77	97	0.8 (0.5–1.2)
Null/slow	117	156	0.9 (0.6–1.2)	77	123	0.6 (0.4–1.0)
Null/rapid	115	127	1.0 (0.7–1.4)	76	117	0.7 (0.5–1.1)

[a] Adjusted for age, physical activity level, alcohol intake, usual number of cigarettes smoked/day, and body size.

Table 5 GSTM-1, NAT2-imputed phenotype, and cigarette smoking exposure and rectal cancer in men

	GST present *n* (cases/controls)	*GST* null *n* (cases/controls)	*GST* present OR (95% CI)[a]	*GST* null OR (95% CI)
Cigarette smoking status				
Never	84/123	88/124	1.0	1.1 (0.7–1.6)
Former	106/112	90/128	1.5 (1.0–2.2)	1.1 (0.7–1.6)
Current (<5 yr)	39/44	63/43	1.3 (0.8–2.2)	2.0 (1.2–3.3)
Pack-years smoked				
None	86/123	88/125	1.0	1.1 (0.7–1.6)
≤20	56/78	53/88	1.1 (0.7–1.7)	0.9 (0.6–1.4)
>20	88/77	101/81	1.8 (1.2–2.8)	1.9 (1.2–2.9)
Total long-term passive smoking (h/wk)				
None	42/59	39/54	1.0	1.0 (0.6–1.8)
1–10	95/126	104/139	1.0 (0.6–1.7)	1.0 (0.6–1.6)
>10	82/82	89/91	1.3 (0.8–2.1)	1.3 (0.8–2.1)
	Slow acetylator	Rapid acetylator	Slow acetylator	Rapid acetylator
Cigarette smoking status				
Never	90/143	74/105	1.0	1.2 (0.8–1.8)
Former	112/128	75/105	1.5 (1.0–2.1)	1.2 (0.8–1.8)
Current (<5 yr)	44/35	53/45	2.0 (1.2–3.4)	1.7 (1.0–2.8)
Pack-years smoked				
None	91/144	75/105	1.0	1.2 (0.8–1.8)
≤20	57/79	45/77	1.2 (0.7–1.8)	0.9 (0.6–1.5)
>20	98/82	84/72	2.1 (1.4–3.2)	1.9 (1.2–3.0)
Total long-term passive smoking (h/wk)				
None	40/65	38/48	1.0	1.3 (0.7–2.4)
1–10	100/142	90/119	1.1 (0.7–1.8)	1.2 (0.7–1.9)
>10	98/89	65/77	1.7 (1.0–2.8)	1.3 (0.7–2.1)

[a] Adjusted for age, physical activity level, alcohol intake, usual number of cigarettes smoked/day (for passive smoking), and body size.

The lack of a detected association in women could be from few women smoking heavily or for long duration, an estrogen effect, or from cigarettes working via a different mechanistic pathway in rectal tumors than in colon tumors. As stated previously, women smoked less than men. Also, our evaluation of smoking risk by estrogen status suggested stronger effects among estrogen negative women, although we were limited in power to detect significant associations.

In our previous study of colon cancer, we detected the strongest associations between colon cancer and cigarette smoking for tumors that were unstable (5) and for *p53* transversions among smokers compared with controls (6). Tumors with a *p53* mutation are more likely to be located distally, whereas MSI is observed most frequently in proximal tumors (28, 29). It is possible that cigarette smoking in rectal cancer involves a *p53* pathway. The lack of association between cigarette smoking and rectal cancer in women may be that cigarette smoking primarily influences a MSI pathway in women mediated by estrogen, given the observed association between estrogen and MSI (30). Unfortunately, tumor marker data are not available at this time to sort out these mechanisms for our rectal cancer cases.

Although this study suggests both similarities and differences between cigarette smoking and rectal and colon cancer, there are reasons to hypothesize that different associations may exist. There are different embryological origins for subsites within the colon; antigen expression differs by tumor site within the colon; and colorectal mucosa differs by its ability to metabolize carcinogens (31). These differences may reflect different susceptibilities to environmental factors by colorectal site or they may indicate that malignant transformations in each segment of the colorectal area occur by different mechanisms (32). A selective increase in cell proliferation in rectal mucosa has been observed in rats fed ethanol. This increase in cell prolif-

Table 6 Combined effect of *GSTM-1* genotype and NAT2-imputed phenotype and cigarette smoking on rectal cancer risk in men

	GST present NAT2 slow *n* (case/control)	*GST* present NAT2 rapid *n* (case/control)	*GST* null NAT2 slow *n* (case/control)	*GST* null NAT2 rapid *n* (case/control)
Cigarette smoking status				
Never	46/72	33/49	44/70	41/54
Former	62/57	40/53	50/71	35/51
Current (<5 yr)	22/19	15/23	22/15	38/22
	OR (95% CI)[a]	OR (95% CI)	OR (95% CI)	OR (95% CI)
Never	1.0	1.2 (0.6–2.1)	1.1 (0.6–1.8)	1.3 (0.7–2.3)
Former	1.9 (1.1–3.2)	1.3 (0.7–2.3)	1.2 (0.7–3.0)	1.1 (0.6–2.1)
Current (<5 yr)	1.9 (0.9–3.9)	1.0 (0.5–2.2)	2.3 (1.1–5.0)	2.5 (1.3–4.8)

[a] Adjusted for age, physical activity level, alcohol intake, and body size.

eration has been shown to increase susceptibility to chemical carcinogens.

ETS or passive smoking has been associated with other forms of cancer (33–35). Few studies have attempted to examine passive smoking and colorectal cancer. One study in Japan showed increased risk in smoking-related cancers, including colorectal cancer, among women as a result of living with someone who smoked in the home (36). Our observation that exposure to cigarette smoke of others is associated with rectal cancer in men supports this previous observation.

Previous studies examining *GST* genotypes and NAT2-imputed phenotypes have been mixed in their association with colorectal cancer. Two larger previous studies, including our own previous examination of tobacco use and *GST* and *NAT2* and colon cancer, have not observed variation in smoking-related risk by genotype (7, 8). However, we observed some difference in rectal cancer risk by cigarette smoking status and *GSTM-1* genotype. Men who were *GST* null experienced a greater risk associated with cigarette smoking. Having a null genotype results in less enzyme activity and ability to detoxify carcinogens that may be present in cigarette smoke. However, it should also be recognized that many genes could influence polycyclic aromatic hydrocarbon and/or modify associations with the genetic variants reported here.

The study has several strengths, including being population based, obtaining long-term tobacco use history, and having an interviewer-administered questionnaire to obtain detailed responses from participants. However, there are limitations. One potentially important limitation is selection bias. As observed in our previous study of colon cancer (37), individuals diagnosed at a more advanced disease stage were less likely to participate in the study. Although in the colon cancer study, we did not observe differences in associations with smoking status by disease stage among men (37), in this study we observe stronger associations for more advanced disease stages at diagnosis. This could be an indication that people who smoke are less likely to have screening and therefore are diagnosed at a more advanced disease stage; adjustment for screening did not alter the stage-specific associations. Stronger associations with more advanced disease also could indicate more aggressive tumors. Given the lower response rates in cases with more advanced disease, it is likely that our estimates of association with rectal cancer are conservative.

In summary, we believe that our data support the association between cigarette smoking and rectal cancer among men but not among women. The association may, in part, be mediated by genotype. Furthermore, there appears to be increased rectal cancer risk from ETS, at least for men. A clearer understanding for the gender differences may be obtained by looking at genetic alterations in tumors.

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References

1. Harvard Report on Cancer Prevention. Causes of human cancer. Smoking. Cancer Causes Control, *7:* S5–S6, 1996.

2. Slattery, M. L., Friedman, G. F., Potter, J. D., Edwards, S., and Ma, K. N. Tobacco use and colon cancer. Int. J. Cancer, *70:* 259–264, 1997.

3. Giovannucci, E., Rimm, E. B., Stampher, M. J., Colditz, G. A., Ascherio, A., Kearney, J., and Willett, W. C. A prospective study of cigarette smoking and risk of colorectal adenoma and colorectal cancer in U. S. men. J. Natl. Cancer Inst. (Bethesda), *86:* 183–191, 1994.

4. Giovannucci, E. An updated review of the epidemiological evidence that cigarette smoking increases risk of colorectal cancer. Cancer Epidemiol. Biomark. Prev., *10:* 725–731, 2001.

5. Slattery, M. L., Curtin, K., Anderson, K., Ma, K. N., Ballard, L., Edwards, S., Schaffer, D., Potter, J., Leppert, M., and Samowitz, W. Associations between cigarette smoking, lifestyle factors, and microsatellite instability in colon tumors. J. Natl. Cancer Inst. (Bethesda), *92:* 1831–1835, 2000.

6. Slattery, M. L., Curtin, K., Ma, K., Schaffer, D., Potter, J., and Samowitz, W. GSTM-1 and NAT2 and genetic alterations in colon tumors. Cancer Causes Control, *11:* 541–548, 2002.

7. Slattery, M. L., Potter, J. D., Samowitz, W., Bigler, J., Caan, B., and Leppert, M. NAT2, GSTM-1, cigarette smoking and colon cancer. Cancer Epidemiol. Biomark. Prev., *7:* 1079–1084, 1998.

8. Gertig, D. M., Stampher, M., Haiman, C., Hennekens, C. H., Kelsey, K., Hunger, D. J. Glutathione *S*-transferase GSTM1 and GSTT1 polymorphisms and colorectal cancer risk: a prospective study. Cancer Epidemiol. Biomark Prev., *7:* 1001–1005, 1998.

9. Deakin, M., Elder, J., Hendricks, C., Peckham, D., Baldwin, D., Pantin, C., Wild, N., Leopard, P., Bell, D. A., Jones, P., Duncan, H., Brannigan, K., Alldersea, J., Fryer, A. A., and Strange, R. C. Glutathione *S*-transferase GSTT1A genotypes and susceptibility to cancer: studies of interactions with GSTM1 in lung, oral, gastric, and colorectal cancers. Carcinogenesis (Lond.), *17:* 881–884, 1996.

10. Edwards, S., Slattery, M. L., Mori, M., Berry, T. D., and Palmer, P. Objective system for interviewer performance evaluation for use in epidemiologic studies. Am. J. Epidemiol., *140:* 1020–1028, 1994.

11. Slattery, M. L., Caan, B., Duncan, D., Berry, T. D., Coates, A., and Kerber, R. A computerized diet history questionnaire for epidemiologic studies. J. Am. Dietetic Assoc., *94:* 761–766, 1994.

12. Zhong, S., Wyllie, A. H., Barnes, D., Wolf, C. R., and Suprr, N. K. Relationship between the GSTM1 genetic polymorphism and susceptibility to bladder, breast, and colon cancer. Carcinogenesis (Lond.), *14:* 1831–1834, 1993.

13. Bell, D. A., Taylor, J. A., Butleer, M. A., Stephens, E. A., Wiest, J., Brubaker, L. H., Kadlubar, F. F., and Lucier, G. W. Genotype/phenotype discordance for human arlyamine *N*-acetyltransferase (NAT2) reveals a new slow-acetylator allele common in African-Americans. Carcinogenesis (Lond.), *14:* 1689–1692, 1993.

14. Terry, M. B., and Neugut, A. I. Cigarette smoking and the colorectal adenoma-carcinoma sequence: a hypothesis to explain the paradox. Am. J. Epidemiol., *147:* 903–910, 1998.

15. Chao, A., Thun, M. J., Jacobs, E. J., Henley, S. J., Rodriquez, C., and Calle, E. E. Cigarette smoking and colorectal cancer mortality. J. Natl. Cancer Inst. (Bethesda), *92:* 1888–1896, 2000.

16. Slattery, M. L., West, D. W., Robison, L. M., French, T. K., Ford, M. H., Schuman, K. L., and Sorenson, A. W. Tobacco, alcohol, coffee, and caffeine as risk factors for colon cancer in a low-risk population. Epidemiology, *1:* 141–145, 1990.

17. Engeland, A., Andersen, A., Haldorsen, T., and Tretli, S. Smoking habits and risk of cancers other than lung cancer: 28 years' follow-up of 26,000 Norwegian men and women. Cancer Causes Control, *7:* 497–506, 1996.

18. Terry, P. D., Miller, A. B., and Rohan, T. E. Prospective cohort study of cigarette smoking and colorectal cancer risk in women. Int. J. Cancer, *99:* 480–483, 2002.

19. Terry, P., Ekbom, A., Lichtenstein, P., Feychting, M., and Wolk, A. Long-term tobacco smoking and colorectal cancer in a prospective cohort study. Int. J. Cancer *91:* 585–587, 2001.

20. Ji, B. T., Dai, Q., Gao, Y. T., Hsing, A. W., McLaughlin, J. K., Fraumeni, J. F., and Chow, W. H. Cigarette and alcohol consumption and the risk of colorectal cancer in Shanghai, China. Eur. J. Cancer Prev., *11:* 237–244, 2002.

21. LeMarchand, L. L., Wilkens, L. R., Kolonel, L. N., Hankin, J. H., and Lyu, L. C. Associations of sedentary lifestyle, obesity, smoking, alcohol use, and diabetes with the risk of colorectal cancer. Cancer Res., *57:* 4787–4794, 1997.

22. Newcomb, P. A., Storer, B. E., and Marcus, P. M. Cigarette smoking in relation to risk of large bowel cancer in women. Cancer Res., *55:* 4906–4909, 1995.

23. Baron, J. A., Gerhardsson de Verdier, and Ekborn, A. Coffee, tea, tobacco, and cancer of the large bowel. Cancer Epidemiol. Biomark. Prev., *3:* 565–570, 1994.

24. Wu, A. H., Paganini-Hill, A., Ross, R. K., and Henderson, B. E. Alcohol, physical activity and other risk factors for colorectal cancer: a prospective study. Br. J. Cancer, *55:* 687–694, 1987.

25. Chiu, B. C., Lynch, C. F., Cerhan, J. R., and Cantor, K. P. Cigarette smoking and risk of bladder, pancreas, kidney, and colorectal cancers in Iowa. Ann. Epidemiol., *11:* 28–37, 2001.

26. Sharpe, C. R., Siemiatycki, J. A., and Rachet, B. P. The effects of smoking on the risk of colorectal cancer. Dis. Colon Rectum, *45:* 1041–1050, 2002.

27. Sturmer, T., Glynn, R. J., Lee, I. M., Christen, W. G., and Hennekens, C. H. Lifetime cigarette smoking and colorectal cancer incidence in the Physicians' Health Study I. J. Natl. Cancer Inst. (Bethesda), *92:* 1178–1181, 2000.

28. Samowitz, W. S., Curtin, K., Ma, K. N., Edwards, S., Schaffer, D., Leppert, M. F., and Slattery, M. L. The prognostic significance of *p53* mutations in colon cancer at the population level. Int. J. Cancer, *99:* 597–602, 2002.

29. Samowitz, W. S., Curtin, K., Ma, K. N., Schaffer, D., Coleman, L., Leppert, M., and Slattery, M. L. Microsatellite instability in sporadic colon cancer is associated with an improved prognosis at the population level. Cancer Epidemiol. Biomark. Prev., *10:* 917–923, 2001.

30. Slattery, M. L., Potter, J. D., Curtin, K., Edwards, S., Ma, K., Anderson, K., Schaffer, D., and Samowitz, W. S. Estrogens reduce and withdrawal of estrogens increases risk of microsatellite instability-positive colon cancer. Cancer Res., *61:* 126–130, 2001.

31. Bulfill, J. A. Colorectal cancer: evidence for distinct genetic categories based on proximal or distal tumor location. Ann. Intern. Med., *113:* 779–788, 1990.

32. Seitz, H. K., and Simanowski, U. A. Alcohol and carcinogenesis. Ann. Rev. Nutr., *8:* 99–119, 1988.

33. Slattery, M. L., Robison, L. M., Schuman, K., French, T. K., Abbott, T., Overall, J., and Gardner, J. Cigarette smoking and exposure to passive smoke are risk factors for cervical cancer. J. Am. Med. Assoc., *261:* 1593–1598, 1989.

34. Tredaniel, J., Boffetta, P., Saracci, R., and Hirsch, A. Environmental tobacco smoke and the risk of cancer in adults. Eur. J. Cancer *29A:* 2058–2068, 1993.

35. Taylor, R., Cumming, R., Woodward, A., and Black, M. Passive smoking and lung cancer: a cumulative meta-analysis. Aust. N Z J. Public Health, *25:* 203–211, 2001.

36. Nishino, Y., Tsubono, Y., Tsuji, I., Komatsu, S., Kanemura, S., Nakatsuka, H., Fukao, A., Satoh, H., and Hisamichi, S. Passive smoking at home and cancer risk: a population-based prospective study in Japanese non-smoking women. Cancer Causes Control, *12:* 797–802, 2001.

37. Slattery, M. L., Edwards, S. L., and Samowitz, W. Stage of colon cancer at diagnosis: implications for risk factor associations? Int. J. Epidemiol., *27:* 382–387, 1998.

Associations between Smoking, Passive Smoking, *GSTM-1*, *NAT2*, and Rectal Cancer

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