Meat and Heterocyclic Amine Intake, Smoking, *NAT1* and *NAT2* Polymorphisms, and Colorectal Cancer Risk in the Multiethnic Cohort Study

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Abstract

Background: N-acetyltransferases (NAT) 1 and 2 are polymorphic enzymes catalyzing the metabolic activation of heterocyclic amines. We investigated the modifying effects of NAT1 and NAT2 polymorphisms on the association of meat consumption, heterocyclic amine intake, and smoking with colorectal cancer risk.

Method: In the Multiethnic Cohort study, participants completed a smoking history and a food-frequency questionnaire at recruitment and a cooked meat module 5 years later to estimate heterocyclic amine intake (2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline). Blood samples were collected from incident cases and age-, sex-, ethnicity-, frequency-matched controls to determine genotypes. For analysis of meat intake and smoking, data were available for 1,009 cases and 1,522 controls; for heterocyclic amine intake analyses, 398 cases and 1,444 controls were available. Multivariate logistic regression models were used to estimate odds ratios.

Results: Smoking was associated with an increased colorectal cancer risk (odds ratio, 1.51; 95% confidence interval, 1.17-1.95) for ≥30 pack-years compared with never smokers (P trend = 0.0004). The association was stronger with presence of the "rapid" compared with the "slow/intermediate" NAT2 genotype (P interaction = 0.003). No significant associations were observed for intakes of red meat, processed meat, and heterocyclic amine, or meat doneness preference, but a dietary pattern high in meat showed a weak positive interaction with the NAT2 genotype (P interaction = 0.05).

Conclusion: The enhanced association between smoking and colorectal cancer risk in subjects with the NAT2 rapid genotype supports a role for NAT2 and tobacco smoke heterocyclic amines in the etiology of colorectal cancer. This study only provides weak support for a similar association with meat heterocyclic amines. (Cancer Epidemiol Biomarkers Prev 2009;18(7):2098–106)

Introduction

Colorectal cancer is the third most common cancer among men and women in the United States and ranks third as a cause of cancer deaths (1). A total of 108,070 new colon cancer cases and 40,740 rectal cancer cases are expected for the year 2008 in the United States (2).

There is considerable evidence in support of an association between smoking and colorectal adenomas (3, 4) and a weaker but suggestive association for colorectal cancer (5). Many (6, 7) but not all (8) of the recent studies investigating the effect of smoking on colorectal cancer reported a positive association, and some, but not all, studies suggested that the effect may be stronger for rectal than for colon cancer (6, 9, 10). A potentially long latency period and inaccurate information on early life smoking behavior has been suggested as one possible explanation for the weaker association of smoking with colorectal cancer compared with adenoma (5). Other well-established risk factors for colorectal cancer are red meat and, particularly, processed meat (11, 12). A recent review rated the evidence for a direct association between consumption of red meat or processed meat and colorectal cancer risk as "convincing" (13).

One of the hypothesized mechanisms to explain an increased colorectal cancer risk with smoking and meat intake is through exposure to heterocyclic amines (14). Their carcinogenic properties have been shown in laboratory animals, including nonhuman primates (15). Heterocyclic amines are present in tobacco smoke and are formed when meat is cooked at high temperatures (16). Heterocyclic amine formation increases with temperature and duration of cooking and varies with the type of meat and the cooking method. DNA adducts have been detected in the colon of volunteers at levels of exposure similar to those obtained through the diet (17).

The metabolic activation of heterocyclic amines is catalyzed by N-acetyltransferases (NAT) 1 or 2 (18, 19), which are coded by genes (*NAT1* and *NAT2*) that are highly polymorphic. Heterocyclic amines can be metabolized more or less efficiently by individuals depending on their *NAT* genotypes.

Based on the underlying mechanisms, it is hypothesized that "rapid" *NAT1* and/or *NAT2* acetylators more readily activate heterocyclic amines to their ultimate carcinogenic forms, thereby amplifying the association of cooked meat and smoking with risk for colorectal cancer. We sought to investigate this hypothesis in a case-control study nested within the Hawaii–Los Angeles Multiethnic Cohort Study.

Material and Methods

Study Design. The Multiethnic Cohort Study was established to investigate lifestyle exposures in relation to various disease outcomes, especially diet and cancer. The respective institutional review boards (University of Hawaii, University of Southern California) approved the study protocol. Recruitment procedures, study design, and baseline characteristics have been reported elsewhere (20). In brief, about 215,000 men and women aged 45 to 75 y at cohort creation in 1993, and five targeted ethnicities (African-American, Japanese-American, Latino, Native Hawaiian, and Caucasian) were enrolled between 1993 and 1996. All study participants initially completed a self-administered comprehensive questionnaire that included a detailed dietary assessment, as well as sections on demographic factors; body weight and height; lifestyle factors other than diet, including smoking history; and family history of cancer. To update selected exposure variables, a four-page questionnaire was administered in 1999 to 2000, which included a module on types of cooking methods for various meat items (see below).

The Rapid Reporting System of the Hawaii Tumor Registry and quarterly linkage to the Los Angeles County Cancer Surveillance Program were used to identify colorectal cancer cases during follow-up. Both registries are members of the Surveillance, Epidemiology, and End Results program of the National Cancer Institute. Annual linkages to the cancer registry of state of California complemented the case ascertainment. A random sample of the cohort stratified by sex and race/ethnicity was selected to serve as potential controls for nested case-control studies. Incident colorectal cancer cases occurring since January 1995 and controls were contacted for donation of a blood sample, that is, for most cases, the blood was obtained after diagnosis. Controls for this study were frequency matched to cases by sex, ethnicity/race, and age. Blood samples were collected at the subjects' homes, processed within 8 h, and stored at -80°C. The participation rate among cases was 74% and varied from 70% in African-Americans to 81% in Latinos. The corresponding rate for controls was 66% and varied from 60% in African-Americans to 71% in Caucasians.

Dietary Assessment. Usual dietary intake was assessed at baseline using a comprehensive quantitative food-frequency questionnaire especially designed and validated for use in this multiethnic population (20, 21). The quantitative food-frequency questionnaire asks about the consumption of >180 food items, including >25 single meat items and mixed dishes, including meat. The quantitative food-frequency questionnaire inquires about the usual frequency based on 8 to 9 categories and amount of food consumed based on three portion sizes per food item. Before calculating food group intake, the food mixtures from the quantitative food-frequency questionnaire were disaggregated to the ingredient level using a customized recipe database. In addition, participants were asked about their usual meat doneness preference.

Approximately 5 y after baseline, a cooked meat module was administered to assess frequency of consumption and degree of outside "brownness" for various meat and fish items cooked by three separate high-temperature methods (pan fried, oven broiled, and grilled or barbecued) during the past year. These data were analyzed in conjunction with the National Cancer Institute Computerized Heterocyclic Amines Resource for Research in Epidemiology of Disease database to estimate intakes of three heterocyclic amine (2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (DiMeIQx); ref. 22).

Dietary exposures used in this analysis were intakes of red meat, processed red meat, and heterocyclic amine (2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline), and preference for well-done meat. In addition, we analyzed a dietary pattern variable (meat and fat pattern), which loaded heavy on meat, discretionary fat, and eggs as an exposure measure in this context. The construction of the dietary patterns in the study population was based on all cohort members and on factor analysis and principal components techniques and has been reported in detail previously (23).

Single-Nucleotide Polymorphism Selection and Genotyping. DNA was extracted from blood lymphocytes using a standard method (QIAamp DNA Blood MINI kit, Qiagen). NAT2 is encoded by an 870-bp gene (*NAT2*) that is polymorphic. The reference *NAT2*4* allele and at least 24 allelic variants have been described that carry one or several nucleotide substitutions. Seven of these, all located in the coding region [G191A (R64Q), C282T, T341C (I114T), C481T, G590A (R197Q), A803G (K268R), and G857A (G286T)], occur with a frequency >1% in at least one ethnic group. Genotyping for these seven variants allows for the detection of 26 of the more common alleles (*NAT2*4; NAT2*5A,B,C,D,E,G,J; NAT2*6A,B,C,E; NAT2*7A,B; NAT2*11A; NAT2*12A,B,C; NAT2*13; NAT2*14A,B,C,D,E,F,G*; ref. 24).

Similarly, we considered the allelic variants identified for *NAT1*. Except for very rare variants (<1%), all (*NAT1*3; NAT1*4; NAT1*10; NAT1*11A,B,C; NAT1*14A,B; NAT1*15; NAT1*17; NAT1*19; NAT1*22*) can be characterized by genotyping eight single-nucleotide polymorphisms [C97T (R33Stop), C190T (R64W), G445A (V149I), C559T (R187Stop), G560A (R187Q), A752T (D251V), T1088A (3'-UTR); ref. 25]. A consensus listing of variant alleles for *NAT2* and *NAT1*, maintained by an international committee, is published.[4]

Individuals with two rapid acetylator alleles (*NAT2*4, NAT2*11A, NAT2*12A,B,C,* and *NAT2*13*) were predicted to have a rapid *NAT2* acetylator phenotype. Individuals with two "slow" acetylator alleles (all other alleles) were predicted to have a slow acetylator

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[4] http://www.louisville.edu/medschool/pharmacology/NAT.html

Table 1. Study participants' characteristics

Characteristic	Cases	Controls	P*
n	1,009	1,522	
Sex			0.01
Men	553 (55) [†]	755 (50)	
Women	456 (45)	767 (50)	
Age	69 (62-74) [‡]	66 (60-72)	<0.01
Ethnicity			0.03
African-American	188 (19)	310 (20)	
Japanese-American	339 (34)	446 (29)	
Native Hawaiian	58 (6)	129 (8)	
Latino	225 (22)	344 (23)	
Caucasian	199 (20)	293 (19)	
BMI	25.9 (23.3-29.2)	25.4 (23.0-28.6)	0.03
Family history of colorectal cancer			<0.01
No	877 (87)	1,390 (91)	
Yes	132 (13)	132 (9)	
Smoking status			<0.01
Never	364 (36)	674 (44)	
Former	477 (47)	620 (41)	
Current	168 (17)	228 (15)	
Ever use of aspirin	381 (39)	563 (38)	0.92
Pack-years of smoking[§]	3.9 (0-19.8)	2.0 (0-19.8)	<0.01
Red meat intake (g/1,000 kcal/d)	18.1 (10.9-25.4)	17.7 (10.4-26.0)	0.73
Processed meat intake (g/1,000 kcal/d)	7.2 (4.0-11.1)	6.7 (3.5-11.0)	0.07
Dietary fiber (g/1,000 kcal/d)	10.9 (8.3-13.8)	11.2 (8.6-14.1)	0.04
Fat (% of energy)	30.4 (25.2-34.7)	30.7 (25.6-35.5)	0.17
Intake of HCA (ng/1,000 kcal/d)[∥]			
Total	176.5 (80.0-326.1)	181.2 (84.9-364.0)	0.25
DiMeIQx	1.9 (0.7-4.0)	1.7 (0.6-4.3)	0.74
MeIQx	28.2 (10.4-57.3)	28.1 (10.0-58.6)	0.95
PhIP	140.9 (62.3-267.4)	146.8 (67.3-296.1)	0.15

Abbreviations: HCA, heterocyclic amine; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxa-aline; DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline; BMI, body mass index.
*χ^2 Test comparing cases and controls for categorical variables or Wilcoxon rank-sum test for continuous variables.
[†] *n*; % in parentheses (all such values).
[‡] Median; interquartile range in parentheses (all such values).
[§] Pack-years was set to 0 for never smokers.
[∥] For analyses with heterocyclic amines, 398 cases and 1444 controls were available.

phenotype. Subjects with one rapid and one slow allele were predicted to have an intermediate phenotype. For the analyses NAT2 slow and intermediate acetylators were collapsed because the rapid acetylator phenotype was specifically found to be associated with an increased colorectal cancer risk in a previous population-based case-control study by our group (26). Individuals with the NAT1*10 allele were predicted to have the ''at-risk'' NAT1 phenotype.

Genotyping of cases and controls was done using a fluorescent 5' endonuclease assay and the ABI PRISM 7900HT Sequence Detection System for allelic discrimination (Taqman, Applied Biosystems). Primers and probes are available on request. For some of the single-nucleotide polymorphisms for which Taqman probes could not be made, the MGB Eclipse Probe System (Nanogen) was used. Amplification reactions were carried out in ABI 9700 thermal cyclers, and allelic discrimination was determined on the ABI PRISM 7900HT Sequence Detection System. The amplification reaction for Nanogen primers and probes consists of PCR master mix from Sigma (catalogue number M4693) and JumpStart Taq. Nucleotide-specific PCR primers and fluorogenic probes were designed using Primer Express (Applied Biosystems) and MGB Eclipse Probe Systems (Nanogen).

In addition to the quality control done by the manufacturers, repeat samples were included for 5% of

participants. Concordance rates >98.7% were obtained for the duplicates. For each of the NAT1 and NAT2 single-nucleotide polymorphisms, <2.3% of the study samples had undeterminable genotypes. The control distributions within ethnic groups were tested for Hardy-Weinberg equilibrium, and all were found to comply with $P > 0.05$.

Statistical Analysis. The distributions between cases and controls were statistically compared by the χ^2 test for categorical variables and the Wilcoxon rank-sum test for continuous variables.

Unconditional logistic regression models were used to compute odds ratios and 95% confidence intervals (95% CI). After exclusion of participants with missing covariate values, a maximum of 1,009 colorectal cancer cases and 1,522 controls were available for the analysis based on the baseline questionnaire. For the analyses with NAT2, 992 cases and 1,493 controls and, for analyses with NAT1, 844 cases and 1,345 controls were available. The analyses of meat doneness preferences were based on a smaller number of participants because of missing information. The analysis of the heterocyclic amine data was based on 398 incident cases and 1,444 controls. Although 90% of the cases and 94% of the controls in the study completed the cooked meat module in the follow-up questionnaire that was administered about 5 y after

Table 2. Main effects of *NAT2* and *NAT1*10* genotypes, meat intake, doneness preference, pack-years of smoking, and heterocyclic amine intake on colorectal cancer risk

	Cases/controls	OR (95% CI)	
		Age-sex-ethnicity adjusted	Multivariate adjusted
NAT2			
Slow	336/497	1	1
Intermediate	414/652	0.91 (0.75-1.10)	0.92 (0.75-1.12)
Rapid	242/344	0.96 (0.75-1.22)	0.99 (0.77-1.27)
P trend*		0.65	0.83
NAT1			
No *10 allele	362/527	1	1
One *10 allele	307/539	0.85 (0.70-1.05)	0.86 (0.70-1.06)
Two *10 allele	175/279	0.98 (0.77-1.26)	1.01 (0.79-1.30)
P trend		0.66	0.83
Red meat intake (g/1,000 kcal/d)			
0 to <10.4	238/380	1	1
10.4 to <17.7	249/381	1.04 (0.83-1.31)	1.01 (0.80-1.28)
17.7 to <26.0	282/381	1.22 (0.97-1.53)	1.11 (0.88-1.41)
26.0+	240/380	1.07 (0.84-1.35)	0.96 (0.74-1.23)
P trend		0.34	0.67
Processed meat intake (g/1,000 kcal/d)			
0 to <3.5	222/380	1	1
3.5 to <6.7	250/381	1.11 (0.88-1.40)	1.04 (0.82-1.32)
6.7 to <11.0	274/381	1.25 (0.99-1.57)	1.13 (0.89-1.44)
110.+	263/380	1.23 (0.97-1.56)	1.08 (0.83-1.39)
P trend		0.05	0.46
Meat and fat pattern (factor score)			
Quartile 1	221/380	1	1
Quartile 2	248/381	1.10 (0.87-1.40)	1.03 (0.81-1.31)
Quartile 3	269/381	1.25 (0.99-1.58)	1.11 (0.87-1.43)
Quartile 4	271/380	1.33 (1.04-1.70)	1.13 (0.86-1.47)
P trend		0.01	0.32
Doneness pref.			
Medium/rare	510/772	1	1
Well done	492/738	1.03 (0.87-1.22)	1.07 (0.90-1.28)
P		0.76	0.43
Pack-years of smoking			
0	364/674	1	1
>0 to <30	461/648	1.38 (1.15-1.66)	1.34 (1.11-1.62)
30+	184/200	1.66 (1.29-2.13)	1.51 (1.17-1.95)
P trend		<0.0001	0.0004
Total HCA (ng/1,000 kcal/d)			
0 to <217.3	131/481	1	1
217.3 to <566.9	146/482	1.17 (0.89-1.54)	1.15 (0.87-1.53)
566.9+	121/481	1.09 (0.81-1.45)	1.03 (0.77-1.39)
P trend		0.55	0.82
DiMeIQx (ng/1,000 kcal/d)			
0 to <1.8	119/481	1	1
1.8 to <6.2	147/482	1.29 (0.97-1.70)	1.25 (0.94-1.67)
6.2+	132/481	1.24 (0.93-1.66)	1.18 (0.88-1.59)
P trend		0.14	0.28
MeIQx (ng/1,000 kcal/d)			
0 to <29.8	126/481	1	1
29.8 to <93.5	141/482	1.14 (0.86-1.50)	1.05 (0.79-1.40)
93.5+	131/481	1.19 (0.89-1.58)	1.09 (0.81-1.47)
P trend		0.23	0.57
PhIP (ng/1,000 kcal/d)			
0 to <171.1	134/481	1	1
171.1 to <460.5	144/482	1.12 (0.85-1.47)	1.11 (0.84-1.47)
460.5+	120/481	1.06 (0.79-1.41)	1.03 (0.77-1.39)
P trend		0.69	0.81

NOTE: Number of cases and controls, odds ratios. Odds ratios were calculated by using logistic regression and adjusted for sex, age at blood draw, and ethnicity (age-sex-ethnicity adjusted) or with additional adjusted for family history of colorectal cancer, body mass index, intake (per 1,000 kcal/d) of dietary fiber, calcium, vitamin D, folic acid, ethanol, physical activity (metabolic equivalents), smoking status and pack-years of smoking, or meat intake, as appropriate (multivariate adjusted).
Abbreviations: OR, odds ratio; pref., preference.
*P for trend is based on Wald test for variable coded as 1 to 3.

baseline, the principle of longitudinal analysis allows counting as cases only those colorectal cancer diagnoses occurring after heterocyclic amine exposure was collected. Indicator variables for quartiles, for main effects, and tertile, for interaction effects, of exposure (meat intake, pack-years of smoking, heterocyclic amine, factor score for meat and fat pattern) were created based on the distribution among controls. Meat intake and heterocyclic

Table 3. Age-sex-ethnicity adjusted odds ratio (95% CI) for meat intake, doneness preference, pack-years of smoking, and heterocyclic amine intake on colorectal cancer risk by *NAT1*10* or *NAT2* genotype

Meat intake or pref.	Acetylator genotype						
	Cases/ controls	*NAT2 slow/ intermediate*	*Cases/ controls*	*NAT2 rapid*	*Cases/ controls*	*NAT1 no*10*	*Cases/ controls*	*NAT1 *10*
Red meat (g/1,000 kcal/d)								
0 to <12.8	244/367	1	83/131	0.89 (0.64-1.24)	120/179	1	162/258	0.98 (0.72-1.34)
12.8 to <22.5	260/393	1.01 (0.80-1.27)	72/105	1.01 (0.71-1.44)	122/181	1.03 (0.74-1.43)	152/276	0.88 (0.64-1.21)
22.5 to <102.7	246/389	1.00 (0.79-1.27)	87/108	1.20 (0.85-1.69)	120/167	1.16 (0.83-1.63)	168/284	0.99 (0.72-1.35)
P interaction*		0.44				0.77		
Processed meat (g/1,000kcal/d)								
0 to <4.7	228/388	1	66/107	0.99 (0.69-1.42)	116/184	1	139/262	0.86 (0.63-1.19)
4.7 to <9.4	270/372	1.22 (0.97-1.54)	79/127	1.00 (0.71-1.40)	121/174	1.08 (0.77-1.51)	169/274	1.01 (0.74-1.39)
9.4 to <88.3	252/389	1.14 (0.91-1.45)	97/110	1.48 (1.06-2.07)	125/169	1.19 (0.86-1.67)	174/282	1.06 (0.77-1.45)
P interaction		0.13				0.93		
Meat and fat pattern (factor score)								
Tertile 1	235/372	1	64/126	0.73 (0.51-1.04)	106/179	1	149/252	1.04 (0.76-1.44)
Tertile 2	251/388	1.03 (0.82-1.30)	79/112	1.04 (0.74-1.48)	123/176	1.21 (0.86-1.69)	161/279	1.03 (0.75-1.42)
Tertile 3	264/389	1.14 (0.90-1.45)	99/106	1.50 (1.08-2.10)	133/172	1.43 (1.01-2.01)	172/287	1.15 (0.83-1.58)
P interaction		0.047				0.48		
Doneness pref.								
Rare/medium	373/563	1	127/194	0.95 (0.72-1.25)	205/263	1	238/411	0.77 (0.60-1.00)
Well done	371/577	0.99 (0.82-1.21)	114/149	1.11 (0.83-1.48)	156/260	0.77 (0.58-1.02)	242/400	0.81 (0.62-1.06)
P interaction		0.42				0.09		
Pack-years								
0	278/475	1	82/187	0.70 (0.51-0.96)	119/223	1	183/360	0.98 (0.73-1.32)
>0 to <30	336/520	1.14 (0.93-1.42)	113/120	1.61 (1.18-2.20)	165/232	1.39 (1.02-1.89)	223/350	1.30 (0.97-1.75)
≥30	136/154	1.48 (1.11-1.97)	47/37	2.02 (1.26-3.24)	78/72	1.98 (1.33-2.96)	76/108	1.33 (0.91-1.96)
P interaction		0.003				0.34		
Total HCA (ng/1,000 kcal/d)								
0 to <217.3	99/363	1	28/111	0.82 (0.50-1.34)	57/202	1	54/228	0.89 (0.58-1.39)
217.3 to <566.9	108/354	1.19 (0.87-1.64)	35/114	1.01 (0.63-1.62)	52/163	1.15 (0.75-1.79)	74/265	1.11 (0.73-1.68)
566.9+	96/374	1.13 (0.81-1.57)	24/100	0.88 (0.52-1.48)	45/139	1.36 (0.86-2.15)	53/281	0.82 (0.53-1.27)
P interaction		0.97				0.29		
DiMeIQx (ng/1,000 kcal/d)								
0 to <1.8	93/374	1	22/100	0.80 (0.47-1.38)	55/192	1	46/237	0.74 (0.47-1.16)
1.8 to <6.2	105/341	1.32 (0.96-1.83)	40/128	1.13 (0.72-1.78)	48/162	1.04 (0.67-1.63)	76/259	1.13 (0.74-1.72)
6.2+	105/376	1.29 (0.93-1.79)	25/97	0.97 (0.58-1.64)	51/150	1.35 (0.86-2.12)	59/278	0.88 (0.57-1.36)
P interaction		0.93				0.22		
MeIQx (ng/1,000 kcal/d)								
0 to <29.8	96/356	1	28/117	0.78 (0.48-1.29)	54/198	1	49/229	0.84 (0.54-1.33)
29.8 to <93.5	102/367	1.07 (0.77-1.47)	35/102	1.08 (0.67-1.74)	60/161	1.35 (0.88-2.08)	67/260	1.01 (0.66-1.55)
93.5+	105/368	1.22 (0.88-1.70)	24/106	0.83 (0.49-1.40)	40/145	1.18 (0.73-1.91)	65/285	1.01 (0.66-1.54)
P interaction		0.497				0.89		
PhIP (ng/1,000 kcal/d)								
0 to <171.1	102/367	1	28/106	0.83 (0.50-1.36)	58/204	1	56/228	0.94 (0.61-1.46)
171.1 to <460.5	106/352	1.13 (0.83-1.55)	35/119	0.94 (0.59-1.50)	51/165	1.13 (0.73-1.76)	71/263	1.05 (0.70-1.60)
460.5+	95/372	1.11 (0.80-1.53)	24/100	0.87 (0.51-1.46)	45/135	1.41 (0.89-2.23)	54/283	0.83 (0.54-1.29)
P interaction		0.98				0.25		

*P For interaction was based on the likelihood ratio test (degrees of freedom, 2) comparing a main effects only model and a model including interaction between genotype and exposure represented as two indicator variables.

amine intakes were analyzed in terms of densities, that is, per 1,000 kcal/d, because densities have been shown to better correlate with reference dietary intake for nutrients (21, 27). Ps for trend were derived from regressions of the quartile (1-4) number as continuous variables. Age-sex-ethnicity–adjusted models were calculated along with multivariate-adjusted models, which contained sex, age at blood draw, race/ethnicity, family history of colorectal cancer, body mass index, intake (per 1,000 kcal/d) of dietary fiber, calcium, vitamin D, folic acid and ethanol, physical activity (metabolic equivalents) and smoking status and pack-years of smoking, or meat intake, as appropriate. Further adjustment for aspirin use, educational attainment, and calcium or folic acid from supplements only marginally changed the odds ratios in the main effects models, and these variables were not included.

The likelihood ratio test was used to determine the significance of interaction with respect to colorectal cancer between genotype (rapid versus slow/intermediate) and exposures represented as one (e.g., cooking preference) or two (e.g., heterocyclic amine) indicator variable(s). The likelihood ratio test was computed as the difference in log likelihoods for a main effects, no interaction model and for a fully parameterized model containing all possible interaction terms for the variables of interest and tested as a χ^2 statistical, with degrees of freedom equal to the number of interaction terms in the latter model. We used polytomous regression and a Wald test to statistically compare the difference in the risk estimates for exposures between colon and rectal cancer.

All statistical analyses were done using SAS statistical software, version 9 (SAS Institute, Inc.), and all statistical tests were two sided.

Results

The characteristics of the study participants are shown in Table 1. About 55% of cases, and 50% of controls were men. Cases were 3 years older than controls on average. Forty-seven percent and 17% of cases were former and current smokers, respectively, compared with 41% and 15% of controls. Number of pack-years of smoking was statistically significantly higher in cases than controls.

Among controls, the frequency for the rapid *NAT2* genotype was 9% in African-Americans, 10% in Native Hawaiians, 63% in Japanese-Americans, 13% in Latinos, and 6% in Caucasians. For *NAT1*, the frequencies for the above listed ethnic groups for the *10 allele were 21%, 12%, 33%, 24%, and 9%, respectively.

Main effect odds ratios for the *NAT1* and *NAT2* phenotype categories, meat intake variables, smoking, heterocyclic amine exposure, and colorectal cancer are shown in Table 2. Neither the *NAT2* nor the *NAT1* phenotypes were associated with colorectal cancer risk. Although a positive association of processed meat intake and colorectal cancer risk was observed in the age-sex-ethnicity–adjusted models, intake was not significantly associated with colorectal cancer risk in the multivariate-adjusted analysis. The meat and fat pattern was positively associated with colorectal cancer in the age-sex-ethnicity–adjusted model but not significantly in the multivariate-adjusted model. Red meat intake, doneness preference, and heterocyclic amine intake were not associated with colorectal cancer risk.

Cigarette smoking was significantly associated with colorectal cancer. In the multivariate model, smokers who smoked <30 pack-years in their lifetime had a 34% higher risk than never smokers, whereas smokers of >30 pack-years had a 51% higher colorectal cancer risk than never smokers. In the subgroup of ever smokers, none of the dietary variables in Table 2 were associated with colorectal cancer risk (data not shown). Separate analyses by sex showed a somewhat stronger positive association between pack-years of smoking and colorectal cancer in women [449 cases, 750 controls; odds ratio, 1.82; 95% CI, 1.16-2.84 for 30+ pack-years of smoking versus never smokers] than men (543 cases, 743 controls; odds ratio, 1.46; 95% CI, 1.05-2.03), but there was no evidence of statistical interaction with sex ($P = 0.55$). All other main effect odds ratios were similar between men and women (data not shown).

Analyses by ethnicity suggested that the positive association with smoking was strongest for Japanese-Americans (335 cases, 438 controls; odds ratio, 2.07; 95% CI, 1.29-3.29) and Caucasians (197 cases, 285 controls; odds ratio, 2.10; 95% CI, 1.28-3.44); however, no statistical interaction was present with race/ethnicity ($P = 0.40$).

Subsite-specific analyses showed a stronger positive association with pack-years of smoking for rectal cancer than colon cancer. The multivariate-adjusted odds ratio (95% CI) for >30 pack-years of smoking was 1.32 (0.99-1.76), with *P* for trend = 0.02, for colon cancer (697 cases) and 2.12 (1.42-3.14), with *P* for trend of <0.0001, for rectum cancer (247 cases; data not shown). The difference between the pack-year risk estimates for colon and rectum cancer was of borderline statistical significance based on a Wald test ($P = 0.06$; degrees of freedom, 2).

Table 3 shows age-sex-ethnicity–adjusted analyses of interactions between *NAT1* or *NAT2* genotypes and meat intake, smoking, or heterocyclic amine exposure. The multivariate-adjusted results are generally similar and are not shown. There was a statistically significant increased colorectal cancer risk observed for the highest intake of processed meat in NAT2 rapid acetylators compared with slow acetylators (odds ratio, 1.48; 1.06-2.07), but the interaction test did not reach statistical significance ($P = 0.13$; Table 3). The corresponding odds ratio was no longer statistically significant in the multivariate-adjusted model (odds ratio, 1.35; 0.96-1.91). A significant interaction was observed with the meat and fat pattern ($P = 0.047$), which remained of borderline significance in the multivariate model ($P = 0.052$); in the latter analysis, however, the odds ratio for individuals in the highest tertile of the meat and fat pattern and fast NAT2 genotype compared with those in the lowest tertile and the slow/intermediate genotype was not significant anymore (odds ratio, 1.32; 0.93-1.88).

A statistically significant interaction was also observed for pack-years of smoking and *NAT2* ($P = 0.003$ for the age-, sex-, and ethnicity-adjusted, and the multivariate-adjusted analysis) on the risk for colorectal cancer (Table 3). The rapid *NAT2* acetylators who smoked >30 pack-years had a higher colorectal cancer risk (odds ratio, 2.88; 2.02/0.70; 95% CI, 1.73-4.82) than slow/intermediate *NAT2* acetylators who also smoked >30 pack-years (1.48; 1.11-1.97) compared with individuals in the same NAT2 genotype category who never smoked. The corresponding odds ratios (95% CIs) for rectum cancer were 5.20 (2.42-11.17) for the *NAT2* rapid acetylators and 1.93 (1.24-3.02) for *NAT2* slow/intermediate acetylators (P for interaction = 0.02) and, for colon cancer, 2.19 (1.23-3.90) and 1.35 (0.98-1.85; P for interaction = 0.04), respectively. None of the remaining associations differed statistically significantly by *NAT2* acetylation status, neither in the sex-age-ethnicity–adjusted (Table 3) nor in the multivariate-adjusted models (data not shown). No statistically significant interaction between pack-years of smoking and *NAT2* was present when we collapsed the intermediate and rapid phenotypes and compared with the slow phenotypes ($P = 0.31$).

No statistically significant effect modification by *NAT1* genotype was present, but the positive association with pack-years of smoking seemed to be somewhat stronger for the *NAT1*10 noncarriers (1.98; 1.33-2.96) than for the *NAT1*10 carriers (1.36; 0.95-1.94; *P* interaction = 0.34; Table 3).

Combined analysis of *NAT1* and *NAT2* did not clarify the associations noted above (data not shown). The odds ratio (95% CI) for at least 30 pack-years of smoking with presence of the rapid *NAT2* phenotype and *NAT1*10 compared with never smokers with slow/intermediate phenotypes in at least one enzyme was 2.15 (1.17-3.95; *P* for interaction = 0.10).

Discussion

In this nested case-control study, the increased colorectal cancer risk associated with smoking was greater among participants with the rapid compared with those with the slow/intermediate *NAT2* genotype, and the interaction was statistically significant ($P = 0.003$). Associations with smoking were stronger for rectal cancer than for colon cancer. A weak positive association with a dietary

pattern that includes a high intake of fat and meat was also suggested, which seemed to be somewhat more marked among rapid *NAT2* acetylators (*P* for interaction = 0.05). No clear evidence was found for main effect or interaction with NAT2 or NAT1 genotype for red and processed meat intake, meat doneness preference, or estimated exposure to heterocyclic amine from meat in a subset of subjects, with regard to colorectal cancer risk.

Evidence suggesting that smoking, especially long-term smoking, may be an important risk factor for colorectal cancer has emerged in the last 15 years, although the data have not been completely consistent (5, 28). Recent International Agency for Research on Cancer and U.S. Surgeon General reports concluded that there was insufficient evidence for including colorectal cancer among tobacco-related malignancies (29, 30). The present study, based on prospectively collected data, provides further evidence for an association between smoking on colorectal cancer risk, particularly among individuals carrying the rapid *NAT2* genotype.

Higher risks for colorectal cancer and colorectal adenoma associated with smoking have been reported with presence of the *NAT2* rapid acetylator genotype (10, 14, 31) but also with the *NAT2* slow acetylator genotype (32-34), whereas other studies have reported no modifications by *NAT2* genotype (8, 35-37). Fewer studies have investigated the modifying effect of *NAT1* genotypes on the smoking and colorectal cancer association (32, 37, 38). Consistent with our findings, one case-control study found a slightly elevated odds ratio with smoking among noncarriers of the *NAT1*10* allele compared with carriers of the *NAT1*10* allele (32). However, two other case-control studies did not observe any effect modification (37, 38). Thus, overall, the findings for *NAT1* or *NAT2* polymorphisms and smoking on colorectal cancer risk are largely inconsistent, with only suggestions of effect modification. However, some studies had low power to formally test for statistical interaction (35-37), few were prospective in design (8, 10, 35, 37, 38), and none, except for a previous study from our group (26), were able to examine the rapid *NAT2* genotype separately. In our data, no increased risk was observed when the rapid acetylator genotype was collapsed with the intermediate genotype and compared with the slow genotype, as most past studies have done. If replicated, these findings would provide support to the role in colorectal cancer of specific heterocyclic amines that are particularly abundant in tobacco smoke, such as 2-amino-9*H*-pyridol[2,3-*b*]indole (39).

The stronger association of smoking with rectal cancer, as compared with colon cancer, in the present study agrees with our earlier findings in a large population based case-control study in Hawaii (9). Current smoking was also reported to be a risk factor for rectal cancer among men but not among women in a Dutch study (40). However, other studies found higher risks associated with smoking for colon cancer, as opposed to rectal cancer (10), or no difference in risk by anatomic subsite (38).

In the present nested case-control study, only a weak association was observed with processed meats and none for red meat. Although red meat has been associated with colorectal cancer in previous studies, the association has been stronger for processed meats (13). We also found that the association with processed meats, partic-

ularly a dietary pattern that includes high intake of fat and meat, seemed somewhat stronger among rapid acetylators. This is consistent with our finding in a recent ecological study based on 27 countries that adjusting for population frequency of the intermediate/fast *NAT2* genotype significantly improves the correlation between per capita meat consumption and colorectal cancer incidence and that both factors combined explain about 80% of the international variation in colorectal cancer incidence (41).

Several other past studies supported the hypothesis that NAT enzymes play a role in the association between colorectal cancer or colorectal adenoma and meat intake (32-35, 42, 43). Some studies observed the positive association in conjunction with smoking (33, 35, 42) or with a combination of *NAT2* intermediate/rapid and *NAT1*10* (32, 43). However, several studies found no or only slight modifications of the associations between meat and colorectal cancer by acetylation genotypes/phenotypes (36, 37, 44, 45). Overall, past studies suggested a somewhat elevated colorectal cancer risk with meat intake among *NAT2* intermediate/rapid acetylator genotypes, but when done, tests for gene-environment interactions most often did not reach statistical significance. Thus, the evidence from past studies has been largely inconsistent.

Studies that have attempted to estimate dietary heterocyclic amine exposure have either reported no associations with intake of 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline, or 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (46, 47), or a significantly increased risk, especially for 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (48, 49), with colorectal adenoma. Studies on colorectal cancer found significantly positive associations with exposure to dietary heterocyclic amine (50, 51) or no association (52). Very few studies to date have investigated the interaction of heterocyclic amine intake and *NAT* polymorphisms on colorectal cancer risk. A case-control study suggested that high intake of 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline in presence of the *NAT1*10* allele increased adenoma risk (53). Another case-control study found a positive association of heterocyclic amine with colon cancer in African-Americans when *NAT1*10* allele was present, whereas the noncarriers of *NAT1*10* were found to be at higher colon cancer risk with high heterocyclic amine intake in Caucasians (54). It should be noted that the present study had limited power to validly estimate odds ratios for heterocyclic amine intake by *NAT* genotypes for the different ethnic groups based on the current follow-up.

Meat preparation methods or preferred brownness or doneness of meat, often used as surrogates for heterocyclic amine intake, were not associated with risk for colorectal adenoma in a Dutch case-control study (42) or colorectal cancer in several other studies (reviewed in ref. 16). In contrast, in a population-based case-control study specifically designed to test this hypothesis in Hawaii, preference for well-done meat, in conjunction with the rapid *NAT2* genotype and high CYP1A2 enzyme activity, was positively associated with colorectal cancer risk (26, 55). This association was especially pronounced in smokers. A mutagen index, calculated as an estimate for exposure to mutagenic or carcinogenic substances based on amount, doneness, and method of

cooking meat, has been suggested to be associated with rectal cancer in men, but the association was neither statistically significant nor modified by NAT2 imputed phenotype and was not present in women (44). In a study on colon cancer, a borderline significant positive association was observed with a mutagen index, which was strongest in *NAT2* intermediate or rapid acetylators (45).

Consistent with our results, studies investigating the associations of *NAT1* or *NAT2* polymorphisms on colorectal cancer risk provided limited support for a main effect association of *NAT1* or *NAT2* genotype with colorectal cancer risk. A recent meta-analysis of these genes and colorectal cancer identified three studies on *NAT1* and 10 studies on *NAT2* (56). The authors calculated a nonsignificant elevation in risk with *NAT1*10* (odds ratio, 1.25; 95% CI, 0.96-1.63) and no association for the *NAT2* rapid acetylator genotype (odds ratio, 1.05; 95% CI, 0.94-1.14). It should be noted, however, that the genotype-phenotype relationship for *NAT1* remains unclear at this point. A lack of main effect for the rapid NAT2 genotype is consistent with the fact that colorectal cancer incidence in Japanese (who are often rapid acetylator) only increased when they became exposed to a meat-rich Western diet, either when they migrated to the United States or more recently in Japan (24).

The present study has several limitations that need consideration. First, we only considered two metabolic enzymes (*NAT2* and *NAT1*). Because additional enzymes are involved in the bioactivation and detoxification of heterocyclic amine, they may also play a role in modifying the associations of smoking or red meat intake and colorectal cancer (26), increasing misclassification for the variables we did measure. In addition, we were unable to study the associations of single variant alleles (except *NAT1*10*) with colorectal cancer because of their low frequencies. Furthermore, multiple testing may have increased the risk for chance findings in our study. For the analysis of heterocyclic amine, power was limited due to a reduced number of incident cases with the available follow-up, especially for analyses of interactions and subgroups. Moreover, although a validation study comparing the heterocyclic amine intake values derived from a meat module similar to the one used in our study with those from a 12-day food diary obtained deattenuated *r* between 0.36 and 0.60 (22), there probably remains a considerable amount of measurement error in estimating heterocyclic amine intake from diet.

The nested case-control study approach is one of the main strengths of this investigation. The assessment of diet and smoking before disease occurrence minimizes recall bias, which is thought to be a serious concern in case-control studies. Furthermore, the study was particularly comprehensive in the number of alleles genotyped and had a large number of subjects with the rapid *NAT2* genotype. The study was population-based and multiethnic, suggesting that the findings should be broadly generalizable to the general population.

In conclusion, this study provides only relatively weak support for a role of heterocyclic amines from meat in colorectal cancer. However, smoking was found to be more clearly associated with colorectal cancer, and its effect was significantly modified by *NAT2* genotype, suggesting that heterocyclic amines in tobacco smoke may play a role in colorectal cancer. Finally, the data also suggested that the association with smoking may be stronger for rectal cancer than colon cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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