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Heterocyclic amine intake, smoking, cytochrome P450 1A2 and *N*-acetylation phenotypes, and risk of colorectal adenoma in a multiethnic population

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Abstract

Objective—Heterocyclic amines (HAA) are animal carcinogens that are present in meat cooked at high temperature and in tobacco smoke. These compounds require activation by cytochrome P450 1A2 (CYP1A2) and *N*-acetyltransferase-2 (NAT2) before they can damage DNA. This study tested the hypotheses that well-done meat and cigarette smoking increase the risk of adenoma, the precursor to most colorectal cancers, especially in individuals with rapid CYP1A2 and rapid NAT2 activities.

Design—An endoscopy-based case–control study of adenoma was conducted among Caucasians, Japanese and native Hawaiians to test this hypothesis. The overall diet and consumption of well-done meat cooked by various high-temperature methods were assessed by interview in 1016 patients with a first adenoma and 1355 controls with a normal endoscopy. A caffeine test was used to assess CYP1A2 and NAT2 activities in 635 cases and 845 controls. Logistic regression was used to account for matching factors and potential confounders.

Results—Smoking was associated with an increased risk of adenoma. Weak non-significant elevated OR were observed for the main effects of HAA intakes or NAT2 activity. However, the combined effects of HAA intakes and NAT2 activity were statistically significant. Subjects in both the upper tertiles of NAT2 activity and HAA intake were at increased risk of adenoma

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compared with subjects in the lower tertiles of NAT2 activity and exposure (2-amino-3,4,8-dimethylimidazo[4,5-f] quinoxaline intake OR 1.70, 95% CI 1.06 to 2.75; 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline intake OR 1.91, 95% CI 1.16 to 3.16; and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine intake OR 2.14, 95% CI 1.31 to 3.49).

Conclusion—The data suggest that rapid *N*-acetylators with high HAA intake may be at increased risk of adenoma.

Colorectal cancer (CRC) remains the third most common malignancy in the USA, with an estimated 143 460 cases expected in 2012.¹ Colorectal adenomas are usually regarded to be the precursor lesions for most CRC. A better understanding of risk factors for adenomas could improve our understanding of CRC aetiology and help develop new means of primary prevention.

There is considerable evidence that smoking increases the risk of adenoma formation, whereas the evidence for an increase in adenoma risk with red and processed meat intake has only been suggestive. One mechanism that might explain the increased adenoma risk with smoking and meat intake is through exposure to heterocyclic amines (HAA), which are present in meat cooked at high temperatures and in tobacco smoke. It has been demonstrated that these compounds are carcinogenic in animals and that they may be bioactivated first by cytochrome P450 1A2 (CYP1A2) in the liver, then by *N*-acetyltransferase-2 (NAT2) in the colon in order for them to damage DNA and, possibly, initiate carcinogenesis. The activity of these enzymes, coded by polymorphic genes, show considerable inter-individual variation, which has led to the hypothesis that any effect of HAA on CRC risk should be more pronounced in individuals with a high activity of these enzymes. A number of variants in the *NAT2* gene have been shown to confer a slow acetylator phenotype,² whereas no specific variant in *CYP1A2* has been shown to affect enzyme activity substantially.³ Past studies, which examined the association of genetic variants in *NAT2*, and/or *CYP1A2*, and some measure of meat or well-done meat intake have only been weakly supportive of the hypothesis.⁴ The activity of these metabolic enzymes can be affected by lifestyle exposures, especially CYP1A2, which is induced by smoking and other factors.^{5,6} Therefore, studies that rely on assessing the phenotype, ie, NAT2 and CYP1A2 activities, may better capture individual risk than studies that infer phenotype from genotype.

In a population-based study in Hawaii with 349 cases and 467 controls, we showed that preference for well-done meat was associated with an 8.8-fold increased CRC risk (95% CI 1.7 to 44.9) among smokers with both a high CYP1A2 activity and the rapid NAT2 genotype.⁷ Similarly, in a case-control study of adenoma or CRC (75 cases, 205 controls), Lang *et al*⁸ found a sixfold increased risk in subjects with both the rapid NAT2 and rapid CYP1A2 phenotypes and preference for well-done meat. In contrast, a case-control study of 146 cases and 228 controls failed to find any modifying effect of NAT2 or CYP1A2 activity, also measured by urinary caffeine metabolites, on the association of HAA with adenoma.⁹

Avoiding HAA exposure may be particularly relevant to Alaska natives and Japanese individuals, two populations at very high risk of CRC,¹⁰ because they have a high

prevalence of the rapid *N*-acetylation phenotype (>90% and >80%, respectively, compared with 8% in Caucasians).¹¹

The aim of this study was to investigate the association of colorectal adenoma with intake of HAA and their food sources, as well as smoking, and to examine the modifying effects of CYP1A2 and NAT2 activity, as measured by caffeine phenotyping.

MATERIALS AND METHODS

The study design and data collection for this case–control study of colorectal adenoma have been described in detail elsewhere.¹² Briefly, one colonoscopy clinic and two flexible sigmoidoscopy screening clinics were used to recruit participants on Oahu, Hawaii. Adenoma cases were identified either as part of the baseline screening flexible sigmoidoscopy examination at the Hawaii site of the Prostate Lung Colorectal and Ovarian Cancer Screening Trial in 1996–2000 or among Kaiser Permanente—Hawaii (KPH) patients who underwent a flexible sigmoidoscopy in the KPH gastroenterology screening clinic (1995–2007) or a colonoscopy in the KPH gastroenterology department (2002–7). Cases were patients 50–75 years of age, of at least 75% Caucasian, 75% Japanese, or any part Hawaiian ancestry, and with a first, pathology-confirmed diagnosis of colorectal adenoma. Controls were selected among patients with a normal colorectum at endoscopy and without a history of previous adenoma, and were individually matched to cases for sex, age at examination (± 3 years), ethnicity, recruitment centre, initial examination clinic and modality (flexible sigmoidoscopy or colonoscopy), and date of screening (± 90 days). The study was approved by the relevant institutional review boards and all participants signed a consent form.

Of the 3457 patients who were eligible for the study, 2371 subjects (1355 controls, participation rate 69.2%; 1016 cases, participation rate 67.8%) agreed to participate. In-person interviews were conducted at the subjects' homes by trained interviewers. The questionnaire included: detailed information on demographics; lifetime history of tobacco, alcohol and aspirin use; a history of recreational sports activities since the age of 18 years; a personal history of various relevant medical conditions; a family history of CRC in parents and siblings; information on height and weight at different ages; and for women, a history of reproductive events and hormone use. The questionnaire also included a 268-item quantitative food frequency questionnaire (QFFQ) augmented with items on the consumption of specific meats (hamburgers, beef, pork, chicken, sausages, bacon) and fish cooked using high-temperature methods (pan-frying, barbecuing or grilling, or broiling) to various levels of doneness (do not eat, rare, medium, well done, very well done). The QFFQ included information on serving size and frequency of foods consumed during the 12 months before screening. This QFFQ has been described and validated previously in this population.^{13,14} The intake of meat/fish cooked at high temperature was calculated by summing the grams per day consumed for all categories of meats and fish cooked by grilling/barbecuing, pan-frying or broiling. Similarly, the intake of well-done meat/fish was computed as the sum of daily intakes of well-done or very well-done meats and fish cooked by these high-temperature methods. To compute intake estimates for 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) and 2-amino-3,4,8-dimethylimidazo[4,5-

f]quinoxaline (Di-MeIQx) and 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine (PhIP), we used values published for meats and fish cooked using various cooking methods and to several doneness levels.^{15–19} All dietary intake values were energy adjusted, including HAA, by the method of residuals after assurance that the model assumptions were met.²⁰

A total of 1480 subjects (635 cases and 845 controls) agreed to the caffeine test, which was performed as described in Le Marchand *et al.*⁷ Using a high-performance liquid chromatography method adapted from that of Butler *et al.*,²¹ we quantified urinary ratios of (1,7-dimethylxanthine + 1,7-dimethyluric acid)/caffeine and 5-acetylamino-6-formylamino-3-methyluracil/1-methylxanthine to determine CYP1A2 and NAT2 activities, respectively. After the exclusion of samples with interfering peaks on the high-performance liquid chromatography, 1452 (621 cases, 831 controls) and 1423 (602 cases, 821 controls) subjects had useable values for NAT2 and CYP1A2 phenotype assessment, respectively. Similar numbers of samples from cases and controls were analysed in each laboratory batch, giving preference to samples from the same matched case–control set. Based on blind duplicates (136 pairs) analysed with the study samples, the coefficient of variation was 14.6% and 17.4% for NAT2 and CYP1A2 activity, respectively.

Logistic regression was used to compute OR (and 95% CI), adjusting for family history of CRC, body mass index 5 years before endoscopy, energy-adjusted total intakes (from foods and supplements) of calcium and folate, pack-years of smoking and alcohol intake, unless otherwise noted. Further adjustment for physical activity and total energy intake did not modify the risk estimates. Linear trends in OR were tested using the median values for each quartile. Conditional logistic regression was used, when possible, to account for the matched sets. However, because not all members of each matched pair agreed to do the caffeine test, unconditional logistic regression was used in analyses that included the caffeine phenotypes, adjusting for the matching factors, as well as the previously mentioned variables. Exposure variables such as urinary ratios, nutrient and food intakes, and pack-years were parameterised as continuous log transformed variables, and categorised into tertiles based on their overall distributions in controls. Linear trends in OR were tested using the median values for each quantile. Interactions were assessed with the likelihood ratio test, comparing the likelihood of a main effects model with a model including both main effects and interaction terms. All statistical analyses were conducted using SAS V.9.1 with a significance level of 0.05 (two-sided).

RESULTS

The main characteristics of study participants are shown by sex in table 1. As expected, adenoma cases in both sexes were more likely to have a first-degree family history of CRC and to be a smoker. In both sexes, cases also had somewhat greater intakes of DiMeIQx, MeIQx, PhIP, total HAA and red and processed meat, and lower intakes of energy-adjusted dietary fibre, calcium and folate (men only), when compared with controls.

Tables 2 and 3 show the multivariate-adjusted OR for adenoma by quartiles of meat and HAA intakes, pack-years of smoking and NAT2 and CYP1A2 activities for men and women, respectively. As expected, pack-years of smoking increased adenoma risk (OR for

increasing quartiles: men, 1.00, 1.46 (95% CI 1.08 to 1.98), 1.25 (95% CI 0.90 to 1.74), 2.12 (95% CI 1.55 to 2.90), *p* for trend <0.0001; women, 1.00, 0.87 (95% CI 0.53 to 1.43), 1.90 (95% CI 1.22 to 2.95), 2.21 (95% CI 1.44 to 3.38), *p* for trend 0.001). There were also non-significant elevated OR (1.2) for processed meat in men, HAA in women and NAT2 in both sexes; however, no statistically significant association was observed. Similarly, no association was found for red meat, processed meat and poultry cooked using high-temperature methods or cooked well-done (see supplementary tables 1 and 2, available online only). Stratified analyses by smoking status (ever; never) or by race (Japanese; Caucasian; Hawaiian) did not reveal significant main effect associations of HAA, NAT2 or CYP1A2 with adenoma risk (data not shown).

Table 4 presents the adenoma OR for the joint effects of intakes of HAA or pack-years of smoking and NAT2 and CYP1A2 activities. The strongest OR were found for subjects in the upper tertile of HAA intake and upper tertile of NAT2 activity, whereas the lowest risk was observed in subjects with the lower tertiles of HAA intake and NAT2 activity (the reference category). While the exposures of HAA intake and NAT2 activity alone did not result in a significantly increased association with risk (tables 2 and 3), significantly elevated OR were observed for subjects in the upper tertiles of NAT2 activity and PhIP intake (OR 2.14, 95% CI 1.31 to 3.49), DiMeIQx intake (OR 1.70, 95% CI 1.06 to 2.75), MeIQx intake (OR 1.91, 95% CI 1.16 to 3.16) and total HAA (OR 1.95, 95% CI 1.19 to 3.18) (table 4). Although these joint effects were significant, we did not observe significant statistical interactions, ie, none of the tests for multiplicative interaction were statistically significant. There was no remarkable joint effect between pack-years of cigarette smoking and NAT2 activity as pack-years appeared to increase adenoma risk among subjects in all tertiles of NAT2 activity. There was no suggestion of joint effects or interactions between CYP1A2 activity and any of these variables (table 4). Similarly, three-way interactions among HAA intake, NAT2 activity and CYP1A2 activity, in all subjects or among ever smokers, did not reveal any clear higher-order interactions. Restricting the analyses involving the CYP1A2 phenotype to smokers did not reveal stronger effects than when all subjects were included.

DISCUSSION

In this large case-control study, we only observed very weak non-significant elevated OR for main effects, but the cumulative effects of HAA intakes and NAT2 activity were statistically significant. Rapid *N*-acetylators who had a high intake of HAAs were at significantly increased risk of adenoma, compared with slow *N*-acetylators with low HAA or smoking exposure. Rapid CYP1A2 activity was not identified as a risk factor for adenoma overall or in any high exposure subgroups. High lifetime smoking levels were significantly associated with risk.

A number of past studies have investigated the combined effects of NAT2 genotype and meat intake or smoking on the risk of colorectal adenoma or cancer. These studies have usually been supportive of an enhanced effect of these exposures on CRC or adenoma risk among subjects with the rapid NAT2 genotype.^{422–25} However, a good number of studies have not shown this.^{26–29} These inconsistencies may be due to methodological differences among studies (eg, variation in power, misclassification on exposure and the grouping of the

intermediate with the rapid genotype). A few studies have specifically estimated dietary intake of various HAA and have found one or more to be associated with adenoma risk.^{929–31} However, studies that looked at the interaction between HAA intake and the *NAT2* or *CYP1A2* genotype on the risk of CRC or adenoma have rarely been supportive of an interaction.^{1213232832–35}

A few studies have measured metabolic phenotypes instead of genotypes, in the hope of better capturing individual risk. A National Cancer Institute study found no association between *CYP1A2* and *NAT2* enzyme activities, as measured by caffeine testing and the risk of adenoma.⁹ This contrasts with our past results on CRC,⁷ and those of Lang *et al*⁸ that suggested a possible enhancing effect of *NAT2* and *CYP1A2* phenotypes on the association between preference for well-done meat and CRC risk. Our current adenoma study supports a similar joint effect of HAA intake with the *NAT2* phenotype, but not with *CYP1A2*.

In this study, care was taken to ensure accurate results for *NAT2* and *CYP1A2* phenotyping. Participants were asked not to eat well-done meat or cruciferous vegetables during the 2 days preceding the caffeine test, because these foods may induce *CYP1A2*.⁷ They were also instructed not to take any caffeine or acetaminophen during the day preceding the test, and to fast for 10 h before the time of the caffeine challenge the following morning and to maintain fasting for another 2 h after dosage. In our experience, these restrictions decrease the intra-individual variability of the metabolic ratio. Our previous data also suggest that one measurement performs reasonably well in characterizing long-term activity for both *NAT2* and *CYP1A2*.⁵⁶ Nevertheless, there was a fair amount of measurement error in the metabolic phenotype, as well as in the dietary intake assessment. However, the error is likely to be non-differential between cases and controls, especially for the biomarkers and, thus, to lead to decreased power rather than to spurious associations. Also, adenomas located in the proximal colon may have been missed in the 69% of controls receiving a flexible sigmoidoscopy, rather than a full colonoscopy, resulting in some misclassification on case–control status. Similarly, such a misclassification would attenuate the risk estimates and be unlikely to create spurious associations. Finally, our power was limited to detect statistical associations in the subset of participants with caffeine metabolic ratios.

Data on *NAT2* and *CYP1A2* from phenotyping was limited to only 1452 and 1423 subjects, respectively, of the 2371 who were interviewed. Compared with those who refused the caffeine test, those with the caffeine metabolic ratio test were less often women (35% vs 41%), native Hawaiian (20% vs 23%), from KPH (82% vs 91%) and recruited through an index colonoscopy (30% vs 48%). However, they were similar with respect to other important variables, such as age, pack-years and intakes of high-temperature meat, well-done meat and alcohol.

In summary, our data suggest that the risk of adenoma, a precursor lesion for CRC, is increased by intake of HAA from well-done grilled/pan-fried meat when combined with a rapid *NAT2* phenotype. These results, if confirmed, would be particularly pertinent to CRC prevention in Alaska natives and Japanese individuals who have a high prevalence of the rapid *N*-acetylation phenotype and very high rates of CRC.¹⁰¹¹

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Significance of this study

What is already known about this subject?

- ▶ HAA are suspected colorectal carcinogens that are formed at the surface of meat and fish when they are cooked at high temperature.
- ▶ These compounds require metabolic activation by CYP1A2 and NAT2 before they can damage DNA.
- ▶ The evidence for an association of HAA with the risk of colorectal neoplasm in humans is limited. Inconsistency in past data may be due to unmeasured individual differences in the ability to bioactivate HAA.

What are the new findings?

- ▶ We assessed the associations of HAA intake and of NAT2 and CYP1A2 activities with the risk of colorectal adenoma in a large case–control study in Hawaii.
- ▶ Although we did not observe statistically significant associations for any factor individually, the combined effects on the risk of adenoma of HAA intakes and NAT2 activity (but not CYP1A2 activity) were statistically significant.
- ▶ Rapid *N*-acetylators who had a high intake of HAA were at significantly increased risk of adenoma.

How might it impact on clinical practice in the foreseeable future?

- ▶ If confirmed, these results would be particularly pertinent to CRC prevention in populations with a high prevalence of the rapid *N*-acetylation phenotype and high CRC rates (eg, Japanese, Alaska natives). They might also be useful in efforts to personalise prevention messages in other populations.

Table 1Characteristics^{*} of cases and controls

	Men		Women	
	Cases (n = 599)	Controls (n = 800)	Cases (n = 365)	Controls (n = 466)
Race/ethnicity [†] , n (%)				
Japanese	183 (30.6)	256 (32.0)	115 (31.5)	154 (33.0)
Caucasian	294 (49.1)	388 (48.5)	165 (45.2)	201 (43.1)
Native Hawaiian	122 (20.4)	156 (19.5)	85 (23.3)	111 (23.8)
Age [†] (years)	59.9 (54.2–66.0)	59.9 (54.5–66.2)	59.8 (54.1–66.8)	60.7 (54.8–67.5)
Family history [‡] , n (%)	86 (14.4)	95 (11.9)	83 (22.7)	74 (15.9)
Smoking status (%)				
Never	206 (34.5)	364 (45.5)	188 (51.5)	288 (61.8)
Past	301 (50.3)	370 (46.3)	128 (35.1)	155 (33.3)
Current	91 (15.2)	66 (8.3)	49 (13.4)	23 (4.9)
Pack-years (smokers only)	26.1 (9.3–47.8)	20.7 (7.9–41.8)	17.2 (6.8–33.8)	10.0 (2.5–28.0)
BMI 5 years ago	26.6 (24.3–30.0)	26.2 (24.2–29.3)	25.4 (22.5–29.2)	24.4 (21.6–27.6)
Ethanol (g/day)	102.9 (0.7–454.1)	41.2 (0.5–271.6)	0.7 (0.2–54.5)	0.7 (0.2–34.1)
Energy intake (kcal/day)	2312 (1778–3096)	2233 (1774–2989)	1698 (1321–2118)	1700 (1292–2154)
Physical activity (MET)	56.1 (47.0–68.5)	57.3 (49.5–68.0)	55.5 (50.3–62.5)	56.8 (50.4–64.5)
Dietary fibre (g/day) [§]	19.4 (15.4–24.3)	20.8 (16.2–26.4)	21.8 (17.6–26.6)	22.5 (18.6–27.4)
Calcium (mg/day) ^{§ ¶}	752.1 (580.3–1021.6)	814.4 (626.5–1122.7)	1094.5 (699.0–1654.0)	1190.2 (785.3–1772.8)
Folate (DFE/day) ^{§ ¶}	758.9 (485.0–1138.2)	917.9 (557.6–1245.4)	998.7 (520.8–1335.5)	938.2 (557.8–1300.0)
Red meat excluding processed meat (g/day)	58.3 (32.3–100.6)	54.9 (28.6–92.8)	36.6 (18.0–59.9)	35.9 (17.2–61.8)
Processed meat (g/day)	27.9 (13.8–49.9)	24.1 (10.5–46.0)	14.9 (6.4–25.8)	14.2 (6.3–26.9)
Poultry (g/day)	20.2 (9.7–38.7)	20.6 (11.6–37.9)	18.3 (9.6–33.8)	16.5 (9.1–31.6)
Fish (g/day)	21.2 (9.9–38.4)	20.9 (10.5–38.7)	14.4 (7.1–25.8)	17.1 (8.5–28.0)
Beef (g/day)	44.3 (24.7–75.6)	41.5 (21.3–69.1)	24.9 (12.9–44.0)	26.3 (12.5–44.0)
Pork (g/day)	10.5 (3.9–22.9)	9.6 (3.3–19.5)	7.3 (2.6–15.8)	6.6 (2.3–15.9)
Di-MeIQx (ng/day) [§]	2.16 (0.59–4.77)	1.89 (0.42–5.00)	1.84 (0.63–4.93)	1.63 (0.53–3.84)
MeIQx (ng/day) [§]	55.4 (20.0–113.9)	53.1 (15.2–116.0)	46.4 (11.7–117.1)	42.1 (8.9–96.6)
PhIP (ng/day) [§]	115.3 (47.8–211.4)	94.5 (40.2–185.3)	95.0 (32.7–209.0)	74.5 (23.7–170.2)
Total HAA (ng/day) [§]	181.0 (81.8–329.1)	167.0 (73.7–308.0)	156.6 (58.0–339.6)	135.8 (42.9–288.9)

BMI, body mass index; DFE, dietary fibre equivalent; Di-MeIQx, 2-amino-3,4,8-dimethylimidazo[4,5-f]quinoxaline; HAA, heterocyclic amine; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-f] quinoxaline; MET, metabolic equivalent; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine.

^{*} Median (IQR), except where noted.

[†] Matching factor.

[‡] Family history of colorectal cancer among parents and siblings.

[§]Energy-adjusted by the method of residuals.

[¶]From foods and supplements.

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Table 2Adenoma OR^{*} (95% CI) by tertile of meat and HAA intakes and NAT2 and CYP1A2 activities among men

	T1 (low)		T2		T3 (high)		P_{trend} Value
	N[†]	OR	N	OR (95% CI)	N	OR (95% CI)	
Poultry (g/day)	209/249	1.00	176/261	0.85 (0.64 to 1.13)	214/290	0.89 (0.67 to 1.18)	0.56
Fish (g/day)	182/246	1.00	188/249	1.03 (0.77 to 1.37)	229/305	1.05 (0.78 to 1.41)	0.74
Red meat excluding processed meat (g/day)	138/218	1.00	198/261	1.02 (0.75 to 1.41)	263/321	1.00 (0.74 to 1.37)	0.97
Processed meat (g/day)	125/223	1.00	198/252	1.27 (0.93 to 1.74)	276/325	1.27 (0.94 to 1.72)	0.23
High-temperature meats (g/day)	133/202	1.00	179/268	0.84 (0.62 to 1.15)	287/330	1.03 (0.76 to 1.41)	0.46
Pan-fried red meat	149/247	1.00	215/249	1.29 (0.95 to 1.76)	235/304	0.99 (0.72 to 1.36)	0.32
Broiled red meat	334/406	1.00	115/179	0.78 (0.58 to 1.04)	150/215	0.83 (0.63 to 1.09)	0.23
BBQ/grilled red meat	172/246	1.00	167/235	0.89 (0.66 to 1.20)	260/319	0.89 (0.67 to 1.20)	0.59
MeIQx [‡] (ng/day)	159/242	1.00	216/275	0.95 (0.70 to 1.29)	224/283	0.91 (0.66 to 1.25)	0.57
PhIP (ng/day) [‡]	154/240	1.00	200/285	0.97 (0.72 to 1.31)	245/275	1.10 (0.81 to 1.48)	0.41
Total HAA [‡] (ng/day)	160/243	1.00	198/280	0.93 (0.69 to 1.27)	241/277	1.01 (0.74 to 1.38)	0.78
Pack-years	207/364	1.00	149/194	1.32 (0.99 to 1.76)	243/242	1.55 (1.00 to 2.40)	0.09
NAT2 activity	134/187	1.00	129/179	0.93 (0.66 to 1.32)	135/173	1.18 (0.81 to 1.70)	0.40
CYP1A2 activity	115/149	1.00	116/179	0.79 (0.56 to 1.13)	163/204	1.00 (0.71 to 1.40)	0.83

BBQ, barbeque; CYP1A2, cytochrome P450 1A2; Di-MeIQx, 2-amino-3,4,8-dimethylimidazo[4,5-f]quinoxaline; HAA, heterocyclic amine; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline; NAT2, *N*-acetyltransferase-2; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine.

* Estimated by conditional logistic regression, adjusting for family history of colorectal cancer, body mass index 5 years ago, energy-adjusted dietary fibre, energy-adjusted total intakes (foods and supplements) of calcium and folate, pack-years of smoking and alcohol intake. Cases and controls were matched for sex, age (within 5-year age groups), race/ethnicity (Japanese, Caucasian or Hawaiian), initial examination modality (flexible sigmoidoscopy or colonoscopy) and clinic and date of screening (within 6 months). Unconditional logistic regression (adjusted for the same variables plus the matching factors) was used for NAT2 and CYP1A2 activities due to the fact that some subjects refused the caffeine test.

[†] Number of cases/number of controls.

[‡] Energy-adjusted by the method of residuals.

Table 3Adenoma OR^{*} (95% CI) by tertile of meat and HAA intakes and NAT2 and CYP1A2 activities among women

	T1 (low)		T2		T3 (high)		P_{trend} Value
	N[†]	OR	N	OR (95% CIs)	N	OR (95% CIs)	
Poultry (g/day)	122/172	1.00	130/162	1.16 (0.82 to 1.65)	113/132	1.23 (0.84 to 1.81)	0.35
Fish (g/day)	153/176	1.00	132/174	0.91 (0.66 to 1.26)	80/116	0.82 (0.56 to 1.21)	0.32
Red meat excluding processed meat (g/day)	148/204	1.00	144/161	1.14 (0.80 to 1.61)	73/101	0.89 (0.58 to 1.37)	0.63
Processed meat (g/day)	151/198	1.00	141/171	0.99 (0.69 to 1.41)	73/97	0.76 (0.49 to 1.17)	0.38
High-temperature meats (g/day)	155/220	1.00	129/155	1.06 (0.76 to 1.50)	81/91	1.06 (0.69 to 1.63)	0.79
Pan-fried red meat	143/181	1.00	123/169	0.79 (0.55 to 1.12)	99/116	0.80 (0.51 to 1.24)	0.43
Broiled red meat	236/297	1.00	86/102	1.03 (0.72 to 1.46)	43/67	0.76 (0.48 to 1.19)	0.25
BBQ/grilled red meat	140/177	1.00	142/183	0.82 (0.59 to 1.14)	83/106	0.72 (0.47 to 1.11)	0.18
MeIQx [‡] (ng/day)	129/179	1.00	109/148	1.05 (0.73 to 1.51)	127/139	1.15 (0.76 to 1.74)	0.50
PhIP (ng/day) [‡]	115/181	1.00	118/139	1.89 (0.96 to 1.99)	132/146	1.31 (0.90 to 1.90)	0.26
Total HAA [‡] (ng/day)	126/179	1.00	107/142	1.10 (0.76 to 1.58)	132/145	1.17 (0.79 to 1.71)	0.46
Pack-years	188/288	1.00	90/113	1.36 (0.93 to 1.98)	87/65	3.07 (1.44 to 6.55)	<0.01
NAT2 activity	60/89	1.00	80/99	1.24 (0.77 to 2.00)	83/104	1.52 (0.92 to 2.50)	0.10
CYP1A2 activity	66/124	1.00	83/96	1.58 (1.02 to 2.43)	59/69	1.61 (1.00 to 2.60)	0.05

BBQ, barbeque; CYP1A2, cytochrome P450 1A2; Di-MeIQx, 2-amino-3,4,8-dimethylimidazo[4,5-f]quinoxaline; HAA, heterocyclic amine; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline; NAT2, *N*-acetyltransferase-2; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine.

* Estimated by conditional logistic regression, adjusting for family history of colorectal cancer, body mass index 5 years ago, energy-adjusted dietary fibre, energy-adjusted total intakes (foods and supplements) of calcium and folate, pack-years of smoking and alcohol intake. Cases and controls were matched on sex, age (within 5-year age groups), race/ethnicity (Japanese, Caucasian or Hawaiian), initial examination modality (flexible sigmoidoscopy or colonoscopy) and clinic and date of screening (within 6 months). Unconditional logistic regression (adjusted for the same variables plus the matching factors) was used for NAT2 and CYP1A2 activities due to the fact that some subjects refused the caffeine test.

[†] Number of cases/number of controls.

[‡] Energy-adjusted by the method of residuals.

Table 4Adenoma OR^{*} (95% CI) for joint effects of HAA intakes and smoking by NAT2 and CYP1A2 activities

HAA source	Tertile cutpoint	NAT2 [†]	N [‡]	OR (95% CI)	Pinteraction	Value	CYP1A2 [§]	N [‡]	OR (95% CI)	Pinteraction	Value
PhIP [¶] (ng/day)	<49	T1	53/101	1.0	0.54		T1	57/87	1.0	0.71	
	50–140		65/85	1.47 (0.91 to 2.39)				53/94	0.74 (0.45 to 1.21)		
	>140		76/90	1.45 (0.90 to 2.33)				71/92	1.01 (0.62 to 1.62)		
	<49	T2	60/93	1.16 (0.71 to 1.88)			T2	59/95	0.80 (0.49 to 1.29)		
	50–140		71/87	1.50 (0.92 to 2.50)				66/90	0.99 (0.61 to 1.60)		
	>140		78/98	1.37 (0.85 to 2.20)				74/90	1.06 (0.66 to 1.71)		
	<49	T3	58/82	1.51 (0.91 to 2.50)			T3	58/91	0.87 (0.54 to 1.42)		
	50–140		69/106	1.34 (0.82 to 2.18)				75/91	1.13 (0.70 to 1.82)		
	>140		91/89	2.14 (1.31 to 3.49)				89/91	1.26 (0.78 to 2.02)		
DiMeIQx [¶] (ng/day)	<0.77	T1	62/104	1.0	0.52		T1	50/103	1.00	0.35	
	0.77–3.27		66/78	1.30 (0.81 to 2.09)				70/87	1.57 (0.98 to 2.54)		
	>3.27		66/94	1.05 (0.66 to 1.68)				61/83	1.31 (0.80 to 2.16)		
	<0.77	T2	71/91	1.22 (0.77 to 1.93)			T2	70/83	1.62 (1.01 to 2.62)		
	0.77–3.27		75/93	1.15 (0.72 to 1.84)				65/99	1.15 (0.71 to 1.85)		
	>3.27		63/94	1.03 (0.64 to 1.66)				64/93	1.28 (0.78 to 2.09)		
	<0.77	T3	60/82	1.32 (0.81 to 2.16)			T3	66/88	1.47 (0.91 to 2.38)		
	0.77–3.27		73/107	1.16 (0.72 to 1.87)				79/87	1.64 (1.02 to 2.64)		
	>3.27		85/88	1.70 (1.06 to 2.75)				77/98	1.47 (0.90 to 2.38)		
MeIQx [¶] (ng/day)	<22.4	T1	53/99	1.0	0.15		T1	48/98	1.0	0.97	
	22.4–82.1		70/90	1.25 (0.77 to 2.02)				66/97	1.28 (0.79 to 2.07)		
	>82.1		71/87	1.31 (0.80 to 2.14)				67/78	1.46 (0.88 to 2.42)		
	<22.4	T2	70/93	1.25 (0.78 to 2.02)			T2	62/88	1.29 (0.79 to 2.10)		
	22.4–82.1		79/89	1.46 (0.90 to 2.37)				64/88	1.25 (0.77 to 2.05)		
	>82.1		60/96	0.98 (0.59 to 1.61)				73/99	1.32 (0.81 to 2.15)		
	<22.4	T3	60/84	1.43 (0.86 to 2.36)			T3	63/87	1.43 (0.88 to 2.34)		

HAA source	Tertile cutpoint	NAT2 [†]	N [‡]	OR (95% CI)	Pinteraction	Value	CYP1A2 [§]	N [‡]	OR (95% CI)	Pinteraction	Value
HAA [¶] (ng/day)	22.4–82.1	T1	64/100	1.17 (0.71 to 1.93)	0.51		T1	77/89	1.49 (0.91 to 2.43)	0.99	
	>82.1		94/93	1.91 (1.16 to 3.16)				82/97	1.51 (0.92 to 2.46)		
	<89.1		54/103	1.0				55/102	1.0		
	89.1–237		66/89	1.28 (0.79 to 2.08)				59/84	1.11 (0.68 to 1.81)		
	>237		74/84	1.48 (0.91 to 2.41)				67/87	1.18 (0.73 to 1.91)		
	<89.1	T2	68/96	1.24 (0.77 to 1.99)			T2	57/85	1.04 (0.64 to 1.69)		
	89.1–237		69/80	1.51 (0.92 to 2.48)				73/96	1.25 (0.78 to 2.00)		
	>237		72/102	1.16 (0.71 to 1.87)				69/94	1.13 (0.70 to 1.82)		
	<89.1	T3	53/77	1.40 (0.83 to 2.35)			T3	57/86	1.11 (0.68 to 1.81)		
	89.1–237		75/109	1.38 (0.85 to 2.24)				75/94	1.27 (0.79 to 2.04)		
	>237		90/91	1.95 (1.19 to 3.18)				90/93	1.52 (0.95 to 2.44)		
Pack-years	0	T1	74/129	1.0	0.66		T1	90/152	1.0	0.08	
	1–19		55/70	1.32 (0.83 to 2.11)				46/77	1.11 (0.70 to 1.77)		
	>19		65/77	1.48 (0.93 to 2.35)				62/60	1.94 (1.22 to 3.08)		
	0	T2	87/156	0.86 (0.57 to 1.29)			T2	79/150	0.87 (0.59 to 1.29)		
	1–19		57/61	1.65 (1.02 to 2.66)				62/80	1.35 (0.87 to 2.08)		
	>19		65/61	1.75 (1.08 to 2.83)				69/58	2.23 (1.41 to 3.53)		
	0	T3	88/133	1.23 (0.80 to 1.89)			T3	72/109	1.23 (0.81 to 1.85)		
	1–19		55/76	1.38 (0.86 to 2.24)				56/51	1.96 (1.22 to 3.16)		
	>19		75/68	2.28 (1.42 to 3.67)				66/84	1.37 (0.88 to 2.13)		

CYP1A2, cytochrome P450 1A2; Di-MeIQx, 2-amino-3,4,8-dimethylimidazo[4,5-f]quinoxaline; HAA, heterocyclic amine; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline; NAT2, *N*-acetyltransferase-2; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine.

* OR and 95% CI are estimated by unconditional logistic regression, adjusted for sex, age, race/ethnicity, initial examination modality (flexible sigmoidoscopy or colonoscopy) and clinic, date of screening, family history of colorectal cancer, body mass index 5 years ago, energy-adjusted dietary fibre, energy-adjusted total intakes (foods and supplements) of calcium and folate, pack-years of smoking and alcohol intake.

[†] Cutpoints are 0.29 and 0.58 based on all controls.

[‡] Number of cases/number of controls.

[§] Cutpoints are 0.87 and 1.08 based on all controls.

[¶] Energy-adjusted by the method of residuals.