Research Article

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Dietary Heterocyclic Amine Intake, *NAT2* Genetic Polymorphism, and Colorectal Adenoma Risk: The Colorectal Adenoma Study in Tokyo

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Abstract

Background: While several studies have provided support for a positive association between meat intake and colorectal neoplasia, the role of heterocyclic amines (HCA), which is hypothesized to underline this relation, has been less consistent. We evaluated the association of HCA intake with colorectal adenoma risk in a case–control study in a middle-aged Japanese population.

Methods: Study subjects were 738 patients with adenoma and 697 controls who underwent total colonoscopy between 2004 and 2005 and responded to self-administered lifestyle and dietary questionnaires. HCA exposure concentration was estimated from meat and fish intake based on an HCA database that was validated against 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) values measured in human hair. Logistic regression models were used to estimate ORs and 95% confidence interval (CI) for the association between HCA and colorectal adenoma risk after adjusting for potential confounders.

Results: High intake of 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ) and total HCA was associated with an increased risk of colorectal adenoma in women but not in men. The multivariate-adjusted OR for the highest versus lowest quartile in women was 2.10 (95% CI, 1.20–3.67; P_{trend} = 0.01) for MeIQ and 1.73 (95% CI, 0.99–3.01; P_{trend} = 0.03) for total HCA. No clear association with PhIP or 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) estimates and no effect modification by *NAT2* acetylation genotype was observed.

Conclusions: This study suggests that high MeIQ and total HCA estimates are positively associated with colorectal adenoma risk.

Impact: The findings add to evidence that HCA may play a role in colorectal carcinogenesis in humans. *Cancer Epidemiol Biomarkers Prev; 24(3); 613–20. ©2015 AACR.*

Introduction

Colorectal cancer is the third most common cancer worldwide and accounted for approximately 10% of all cancer cases diagnosed in 2008 (1). Japan has experienced a rapid increase in the incidence of colorectal cancer in the last few decades, particularly of colon cancer. It has been argued that this increase is primarily due to Westernization of the diet, including increased intake of meats (2). High intake of meat, particularly red and processed meat, has been associated with an increased risk of colorectal cancer in several epidemiologic studies (3), and collectively, this evidence has led to the conclusion that red and processed meat are convincing causes of colorectal cancer (4). However, it remains unclear whether this association is mediated by constituents in meat itself or by exposure to other mutagenic compounds produced during cooking, like heterocyclic amines (HCA). HCA compounds are formed from the reaction of creatine/creatinine, amino acids, and sugars at high temperatures (5, 6), and their concentrations vary by type of meat and cooking method and by the temperature and duration of cooking (6, 7). Three commonly detected types of HCA in cooked meats are 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), and 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (Di-MeIQx; ref. 7).

Most HCAs are potent mutagens in bacterial assays and induce tumors in different tissues, including the gastrointestinal tract, in laboratory animal experiments (8–10). HCA-specific DNA adducts have also been detected in some specimens of human colon tissue samples, suggesting that HCA exposure may also be related to human cancer risk (11, 12), but epidemiologic data for HCA intake and colorectal neoplasia in humans are inconsistent. Higher intake of total or type-specific HCA estimated from cooked meat and fish was associated with increased risk of colorectal cancer or its precursor colorectal adenoma in some U.S. (13–17) and European studies (18, 19), but not others (20–23). A Swedish study found no association between overall HCA intake and colon or rectal cancer but suggested carcinogenic potential at extreme high intake (24). Another U.S. study found a suggestive positive

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association of distal colon adenoma with meat-derived mutagenicity, an overall measure of mutagenic activity in cooked meat, but not with other HCAs (25).

N-Acetyltransferase (NAT) 2 is an important enzyme in the activation of HCA and polycyclic aromatic hydrocarbons (PAH) through *O*-acetylation. Combinations of SNP in *NAT2* result in enzymes with slow or rapid acetylation. Rapid acetylators might more readily activate HCAs to their ultimate carcinogenic forms, increasing the risk of colorectal cancer related to these carcinogens (26, 27).

Most data on the relation between HCA estimates and colorectal neoplasia are from Western populations, which tend to consume more red and processed meat and less fish, and no studies in Asian populations have appeared. Fish and poultry under certain cooking conditions are also considerable sources of HCA intake (25, 28). The higher intake of fish than animal meat among Japanese and preference for chopped and stir-fried as well as grilled cooking methods (28) suggests that HCA profile might differ between Japanese and Western populations.

Here, we investigated the association of meat, fish, and meat- and fish-derived HCA intake with colorectal adenoma among middle-aged and elderly Japanese men and women in the Tokyo Colorectal Adenoma Study. We also evaluated the influence of *NAT2* acetylation genotype on the association of HCA and colorectal adenoma risk.

Materials and Methods

Study population

The Colorectal Adenoma Study in Tokyo was a case–control study that examined lifestyle factors and genetic susceptibility in the risk of colorectal adenoma. Participants were examinees who underwent magnifying colonoscopy with dye spreading in a cancer screening program by the Research Center for Cancer Prevention and Screening, National Cancer Center, between February 2004 and February 2005. Details of the methods have been described elsewhere (29, 30). Briefly, men and women ages 50–79 and 40–79 years, respectively, who underwent total colonoscopy from the anus to the cecum and had no history of colorectal adenoma, any malignant neoplasm, ulcerative colitis, Crohn disease, familial adenomatous polyposis, carcinoid tumor, or colectomy were considered eligible. Among 3,212 consecutive examinees, 2,234 met the above conditions. On the basis of the pit pattern classification of colorectal lesions, 526 men and 256 women had at least one adenoma and were thus included as adenoma cases. Of the remaining 1,452 examinees, 482 men and 721 women were free from other benign lesions (e.g., hyperplastic polyps, inflammatory polyps, and diverticula) and were considered potential controls. Because of fewer potential control than cases males, all potential male controls were included in the study, whereas 256 potential female controls were frequency-matched to the cases in 5 age categories (40–49, 50–54, 55–59, 60–64, and $\geq$65 years of age) and 2 screening periods (first or second half). Thus, the study included 782 cases and 738 controls. The screening period was matched because standard operating procedures were improved during the first half period after establishment of the Research Center, which might have influenced diagnostic accuracy (30). In this analysis, we excluded 42 cases and 32 controls with extreme energy intake (top and bottom 2.5% of total energy intake distribution) and 2 cases and 9 controls with missing values for covariates, leaving 738 cases and 697 controls for final analysis. All examinees gave written informed consent for use of screening data for medical research purposes. The study protocol was approved by the institutional review board of the National Cancer Center, Tokyo, Japan.

Lifestyle and dietary factor assessment

Participants completed a self-administered questionnaire survey before screening, which inquired about demographic and lifestyle characteristics, personal and family medical history, supplemental drug use, and reproductive factors (in women). They also completed a 138-item food frequency questionnaire (FFQ) with prespecified standard portion sizes and 9 intake frequency categories for most food items. The options for portion size were less than half, the same as, and more than 1.5 times the standard portion size. The options for frequency of intake of food items were <1 time/mo, 1–3 times/mo, 1–2 times/wk, 3–4 times/wk, 5–6 times/wk, 1 time/d, 2–3 times/d, 4–6 times/d, and $\geq$7 times/d. Intake amount was calculated by multiplying intake frequency by the standard portion size and the relative portion size for each food item in the FFQ. The FFQ inquired about the consumption of 18 meat items and 19 fish/seafood items: 4 beef dishes (steak, grilled beef, stir-fried beef, and stewed beef), 6 pork dishes (stir-fried pork, deep-fried pork, stewed pork in the Western style, stewed pork in the Japanese style, pork in soup, and pork liver), 3 processed meat products (ham, sausage or Wiener sausage, and bacon), chicken liver, 4 poultry items (grilled chicken, stir-fried chicken, stewed chicken and deep-fried chicken), 8 fresh fish (salmon, skipjack/tuna, Japanese amberjack, cod/flatfish, sea bream, horse mackerel/sardine, saury/mackerel, and eel), 4 non-fish seafood items (squid, octopus, shrimp, and clam), 4 salted and dried fish products (salted fish, dried fish, dried whitebait, and salted fish roe), 1 other fish product (canned tuna), and 2 fish paste products (chikuwa and kamaboko). The deattenuated correlation coefficient between energy-adjusted meat and fish/seafood intake estimated from the FFQ and 4-day dietary records for men and women was 0.70 and 0.36 for meat and 0.69 and 0.57 for fish/seafood intake, respectively (31). Consumption of specific HCA from meat and fish was estimated using previous methods (28, 32, 33). The intake of total or specific HCA estimated from the FFQ was compared against PhIP levels measured in human hair (33). Spearman rank correlation coefficients between HCA from the FFQ and PhIP level in hair were 0.33 for PhIP, 0.33 for 2-amino-3,4-dimethylimidazo[4,5-*f*]quinolin (MeIQ), and 0.32 for total HCA in men. The corresponding values in women were 0.37 for PhIP, 0.55 for MeIQ, and 0.42 for total HCA.

Blood collection and genotyping

Fasting venous blood was drawn into a vacutainer tube with ethylenediaminetetraacetic acid before any colonoscopy screening procedures. The vacutainer tubes were centrifuged to obtain plasma and buffy coat layer, and the samples were stored at −80°C until analysis. DNA was extracted from white blood cells in our laboratory using a FlexiGene DNA kit (Qiagen). Genotyping was done with laboratory personnel blinded to case and control status. We determined 3 SNPs in the *NAT2* gene [T341C (rs1801280), G590A (rs1799930), and G857A (rs1799931)] using TaqMan SNP Genotyping Assays (Applied Biosystems). Acetylation genotype was inferred using these 3 SNPs, with individuals with no, 1, and 2 or more variant alleles categorized as rapid, intermediate, and slow acetylation genotypes, respectively.

Statistical analysis

Comparison of means, medians, and proportions between cases and controls was done by the *t* test, Wilcoxon rank-sum test, and χ^2 test, respectively. Intakes of HCA and other dietary variables were transformed to the natural log scale and adjusted to total energy intake by the residual regression method. Subjects were divided into quartile categories according to intakes of HCA among controls of each sex. Unconditional logistic regression models were applied to estimate the ORs and 95% confidence interval (CI) for the association between exposure variables and colorectal adenoma, using the lowest quartile as reference. Because fish intake, which was the largest contributor to both total and individual HCA intake in our study, was significantly different in men and women, we performed all analysis for men and women separately. Factors associated with colorectal adenoma in our data set or associated with colorectal cancer or adenoma in the literature were considered as covariates as follows: age (40–49, 50–54, 55–59, 60–64, ≥65 years), screening period (first or second half), cigarette smoking (never, ≤20, 21–40, and ≥40 pack-years), alcohol consumption (never, past, <150, 150–299, ≥300 g/wk), physical activity (MET-h/d, quartile), body mass index (<21.0, 21.0–22.9, 23.0–24.9, ≥25.0 kg/m^2), family history of colorectal cancer, nonsteroidal anti-inflammatory drug use, and total energy intake and energy-adjusted dietary intake of isoflavone, folate, and fiber (all quartiles). Females were further adjusted for age at menarche (<12, 12–13, ≥14 years), menopausal status (pre- or postmenopausal), and use of female hormones (user or nonuser). Ordinal scores of 0–3 were assigned to quartile categories of intake to assess the trend of an association. Interactions between HCA estimate and *NAT2* acetylation genotype were evaluated using the log-likelihood ratio test by comparing the models with and without the interaction terms. Statistical significance was declared if the 2-sided *P* value was <0.05 or if the 95% CI did not include unity. All statistical analyses were performed using SAS version 9.3 (SAS Institute Inc.).

Results

Selected characteristics of cases and controls are summarized by sex in Table 1. In men, cases were older, had higher BMI and alcohol intake, and were less likely to use NSAIDs than controls. In women, cases had a higher proportion of parental colorectal cancer history than controls but did not differ with respect to other lifestyle factors. With regard to food and nutrient intake, total energy, red meat, processed meat, poultry, and total and type-specific HCA tended to be statistically significantly higher in female cases than in controls but did not differ between male cases and controls. There were also no appreciable differences in fish, fiber, folate, or isoflavone intake between cases and controls of either sex, except that isoflavone intake was higher in female controls than cases. Overall, except for processed meat, red meat, poultry, and fish were all moderately correlated ($r = 0.32$–0.65) with HCA intake in both men and women (Supplementary Table S1). Fish was the largest contributor to the intake all 3 HCA in both sexes, at approximately 50% of PhIP, 40% of MeIQx, and 56% of MeIQ in men and 49% of PhIP, 43% of MeIQx, and 59% of MeIQ intake in women (Supplementary Table S2).

We adjusted for covariates using 3 models. The OR in the matching factors only-adjusted (first) model was slightly attenuated when we further adjusted for lifestyle factors (second model).

Table 1. Characteristics of adenoma case and control subjects

	Men			Women		
	Case (*n* = 498)	**Control (*n* = 453)**	***P***	**Case (*n* = 240)**	**Controls (*n* = 244)**	***P***
Age[a], y	61.3 (5.9)	60.0 (5.7)	0.001	59.8 (6.6)	59.6 (6.4)	0.68
BMI, kg/m^2	24.0 (2.6)	23.4 (2.7)	0.001	22.6 (3.2)	22.2 (2.8)	0.15
Alcohol intake, g/wk	228.9 (231.8)	185.2 (202.9)	0.002	39.1 (89.1)	44.4 (104.2)	0.54
Physical activity, METS-h/d	36.1 (8.9)	35.7 (6.6)	0.46	38.1 (9.7)	37.9 (9.2)	0.83
Smoking history, *n* (%)			<0.001			0.11
Never smoker	139 (27.9)	149 (32.9)		192 (80.0)	213 (87.3)	
Past smoker	231 (46.4)	242 (53.4)		30 (12.5)	23 (9.4)	
Current, ≤20 cig/d	73 (14.7)	39 (8.6)		17 (7.1)	7 (2.9)	
Current, >20 cig/d	55 (11.0)	23 (5.1)		1 (0.4)	1 (0.4)	
Parental colorectal cancer, *n* (%)	44 (8.8)	35 (7.7)	0.56	33 (13.8)	18 (7.4)	0.02
NSAID use, *n* (%)	20 (4.0)	38 (8.4)	0.006	12 (5.0)	15 (6.1)	0.58
Menarche age, y	—	—		13.3 (1.5)	13.2 (1.4)	0.97
Postmenopause, *n* (%)	—	—		216 (90.0)	218 (89.3)	0.81
Hormone use, *n* (%)	—	—		23 (9.6)	29 (11.9)	0.41
Dietary intake, median (IQR)						
Total energy, kcal/d	1,930 (1,643–2,350)	1,924 (1,605–2,269)	0.14	1,917 (1,550–2,261)	1,792 (1,518–2,122)	0.03
Fiber, g/d	11.1 (8.5–14.1)	11.5 (8.9–15.0)	0.06	15.3 (12.3–18.2)	15.6 (12.7–18.2)	0.45
Folate, μg/d	361(271–444)	356 (283–463)	0.34	447 (356–555)	463 (374–567)	0.15
Isoflavone, mg/d	33.2 (20.3–52.9)	35.1 (21.9–56.2)	0.10	41.2 (24.1–63.5)	44.9 (30.4–63.0)	0.07
Total meat, g/d	43.7 (26.3–65.9)	43.6 (27.6–66.8)	0.98	48.9 (28.8–74.9)	40.4 (24.4–64.3)	0.02
Red meat, g/d	27.9 (14.8–43.2)	27.0 (15.7–44.3)	0.89	28.5 (15.8–46.7)	24.4 (14.8–39.9)	0.07
Processed meat, g/d	3.0 (0.6–8.3)	3.6 (0.8–7.8)	0.53	4.8 (1.9–9.9)	3.7 (0.8–9.0)	0.04
Poultry, g/d	7.9 (3.5–14.5)	9.1 (3.5–15.8)	0.52	9.3 (3.1–17.8)	8.0 (1.3–15.8)	0.05
Fish, g/d	61.5 (39.7–88.1)	58.4 (39.8–82.8)	0.62	68.5 (48.0–91.5)	72.6 (47.4–98.7)	0.56
PhIP, ng/d	17.7 (11.7–26.5)	18.3 (10.9–27.1)	0.68	20.2 (13.7–30.4)	17.8 (12.3–26.0)	0.03
MeIQx, ng/d	4.0 (2.7–5.7)	4.0 (2.5–6.0)	0.67	4.5 (3.0–6.7)	4.2 (2.7–5.8)	0.04
MeIQ, ng/d	3.4 (2.4–5.1)	3.6 (2.3–5.1)	0.90	3.9 (2.8–6.0)	3.5 (2.2–5.2)	0.005
Total HCA, ng/d	28.9 (19.4–44.4)	30.0 (17.8–44.5)	0.71	33.0 (21.9–48.5)	28.0 (18.2–42.7)	0.02

Abbreviation: IQR, interquartile range.

[a]Values are means (SD) where not mentioned.

Table 2. Multivariate-adjusted OR and 95% CI of colorectal adenoma according to meat type

	Men			Women		
Quartile (Q)	**Intake[a]**	**N[b]**	**OR (95% CI)[c]**	**Intake[a]**	**N[b]**	**OR (95% CI)[c]**
Total meat						
Q1	16.5	129/113	1.00 (Reference)	15.6	49/61	1.00 (Reference)
Q2	35.6	120/113	0.95 (0.65–1.38)	33.1	52/61	1.05 (0.60–1.83)
Q3	53.1	129/113	1.09 (0.75–1.59)	52.2	58/61	1.00 (0.58–1.74)
Q4	85.9	120/114	1.04 (0.71–1.53)	83.3	81/61	1.58 (0.92–2.71)
P_{trend}			0.68			0.11
Red and processed meat						
Q1	10.9	131/113	1.00 (Reference)	10.7	60/61	1.00 (Reference)
Q2	26.5	112/113	0.90 (0.61–1.32)	24.5	47/61	0.75 (0.43–1.31)
Q3	40.3	128/113	1.15 (0.79–1.68)	38.5	55/61	0.77 (0.45–1.32)
Q4	68.4	127/114	1.12 (0.76–1.64)	61.0	78/61	1.23 (0.72–2.09)
P_{trend}			0.35			0.44
Red meat						
Q1	7.8	133/113	1.00 (Reference)	8.1	56/61	1.00 (Reference)
Q2	21.5	107/113	0.84 (0.58–1.24)	19.7	45/61	0.71 (0.41–1.25)
Q3	34.1	141/113	1.16 (0.80–1.68)	33.4	60/61	0.88 (0.51–1.51)
Q4	60.5	117/114	1.00 (0.68–1.47)	52.7	79/61	1.33 (0.78–2.26)
P_{trend}			0.59			0.21
Processed meat						
Q1	0.0	126/113	1.00 (Reference)	0.0	47/61	1.00 (Reference)
Q2	2.3	148/113	1.21 (0.84–1.75)	2.2	46/61	1.11 (0.62–1.98)
Q3	5.3	92/113	0.83 (0.56–1.22)	5.1	77/61	1.82 (1.05–3.17)
Q4	13.0	132/114	1.13 (0.77–1.64)	13.0	70/61	1.62 (0.94–2.82)
P_{trend}			0.99			0.03
Poultry						
Q1	0.0	127/113	1.00 (Reference)	0.0	41/61	1.00 (Reference)
Q2	5.8	155/113	1.25 (0.86–1.80)	4.9	68/61	1.63 (0.94–2.85)
Q3	11.6	106/113	0.94 (0.64–1.39)	10.6	56/61	1.38 (0.78–2.45)
Q4	21.1	110/114	0.93 (0.63–1.36)	24.8	75/61	1.75 (1.00–3.06)
P_{trend}			0.41			0.11
Fish						
Q1	29.8	125/113	1.00 (Reference)	32.5	56/61	1.00 (Reference)
Q2	50.1	110/113	0.84 (0.58–1.23)	59.1	73/61	1.23 (0.72–2.08)
Q3	70.5	120/113	0.90 (0.62–1.32)	83.4	60/61	1.01 (0.59–1.73)
Q4	106.2	143/114	1.03 (0.71–1.49)	121.1	51/61	0.96 (0.55–1.68)
P_{trend}			0.79			0.72

[a]Median (g/d).

[b]Cases/controls.

[c]Adjusted for age, screening period, smoking, alcohol consumption, body mass index, physical activity, family history of colorectal cancer, and NSAID use. Further adjusted for age at menarche, menopausal status, and current use of hormones in women.

Further addition of total energy intake and other dietary factors in the third model did not change the estimates appreciably and hence the results for the second model are shown, whereas the results for the other 2 models are presented in Supplementary Tables S3 to S6.

The multivariate-adjusted ORs and 95% CIs of colorectal adenoma risk according to quartiles of meat intake are shown in Table 2. Intake of total meat, red and processed meat, red meat, chicken, and fish was not associated with colorectal adenoma risk in either men or women. In contrast, the higher intake of processed meat was positively associated with colorectal adenoma risk in women ($P = 0.03$) but not in men. No appreciable change in the risk estimates was observed when we further mutually adjusted for meat variables in all of the models above (Supplementary Tables S3 and S4).

The multivariate-adjusted ORs and 95% CIs of colorectal adenoma risk according to quartiles of HCA are shown in Table 3. The estimates of individual or total HCA showed no evidence of any association with colorectal adenoma risk in men. In women, however, the higher quartile of MeIQ and total HCA, but not PhIP or MeIQx, showed a significantly increased risk of colorectal adenoma. The *P* value for interaction for the HCA–colorectal adenoma association by sex was 0.053. The number of proximal, distal, and rectal adenoma cases was 249, 184, and 56 in men and 135, 77, and 25 in women, respectively. Respective ORs (95% CI) for the upper compared with the lower tertile (reference) according to intake of fish, total meat, and total HCA among males were 0.97 (0.65–1.44), 1.01 (0.67–1.51), and 0.93 (0.62–1.39) for the proximal colon; 0.94 (0.60–1.46), 1.32 (0.83–2.09), and 1.09 (0.69–1.73) for the distal colon; and 0.68 (0.33–1.40), 1.71 (0.80–3.66), and 0.73 (0.33–1.62) for the rectum. Corresponding values for females were 0.76 (0.43–1.35), 1.54 (0.86–2.75), and 1.62 (0.92–2.87) for the proximal colon; 0.91 (0.44–1.89), 1.62 (0.82–3.20), and 1.06 (0.52–2.19) for the distal colon; and 3.88 (0.89–16.9), 1.50 (0.45–5.04), and 1.70 (0.47–6.18) for the rectum.

A total of 74 cases and 71 controls could not be genotyped and were therefore excluded from the genotype analysis. Genotype frequency among controls was in Hardy–Weinberg equilibrium for all 3 SNPs. The associations between *NAT2* acetylation genotype and colorectal adenoma risk and between total HCA and MeIQ estimate and colorectal adenoma risk stratified by *NAT2* acetylation genotype are summarized in Tables 4 and 5, respectively. *NAT2* acetylation genotype was not related to colorectal adenoma risk in either sex (Table 4). Furthermore, *NAT2*

Table 3. Multivariate-adjusted OR and 95% CI of colorectal adenoma according to HCA estimates

	Men			Women		
Quartile (Q)	**Intake[a]**	**N[b]**	**OR (95% CI)[c]**	**Intake[a]**	**N[b]**	**OR (95% CI)[c]**
PhIP						
Q1	7.4	108/113	1.00 (Reference)	7.5	50/61	1.00 (Reference)
Q2	14.1	150/113	1.33 (0.91–1.93)	15.0	46/61	0.74 (0.42–1.32)
Q3	22.3	121/113	1.09 (0.74–1.60)	22.5	67/61	1.08 (0.62–1.88)
Q4	33.4	119/114	1.02 (0.69–1.50)	33.4	77/61	1.43 (0.83–2.45)
P_{trend}			0.77			0.09
MeIQx						
Q1	1.8	105/113	1.00 (Reference)	2.2	48/61	1.00 (Reference)
Q2	3.2	146/113	1.33 (0.91–1.94)	3.5	56/61	1.02 (0.58–1.78)
Q3	4.9	137/113	1.26 (0.86–1.85)	5.0	55/61	0.97 (0.56–1.70)
Q4	7.4	110/114	1.01 (0.68–1.50)	7.0	81/61	1.58 (0.92–2.73)
P_{trend}			0.97			0.10
MeIQ						
Q1	1.5	118/113	1.00 (Reference)	1.6	37/61	1.00 (Reference)
Q2	2.9	143/113	1.21 (0.84–1.76)	2.9	58/61	1.42 (0.80–2.53)
Q3	4.4	114/113	0.95 (0.65–1.40)	4.3	64/61	1.73 (0.97–3.08)
Q4	6.2	123/114	0.94 (0.64–1.37)	6.0	81/61	2.10 (1.20–3.67)
P_{trend}			0.47			0.01
Total HCA						
Q1	12.3	108/113	1.00 (Reference)	12.7	43/61	1.00 (Reference)
Q2	23.4	153/113	1.43 (0.98–2.08)	23.8	50/61	1.04 (0.58–1.86)
Q3	35.8	115/113	1.05 (0.71–1.55)	36.4	73/61	1.44 (0.83–2.51)
Q4	53.6	122/114	1.02 (0.69–1.50)	54.3	74/61	1.73 (0.99–3.01)
P_{trend}			0.64			0.03

[a]Median (ng/d).

[b]Cases/controls.

[c]Adjusted for age, screening period, smoking, alcohol consumption, body mass index, physical activity, family history of colorectal cancer, and NSAID use. Further adjusted for age at menarche, menopausal status, and current use of hormones in females.

acetylation genotype did not exert any noticeable effect modification on the association between HCA or MeIQ estimates and colorectal adenoma risk (Table 5) in either men ($P_{interaction} = 0.81$ and 0.60 for HCA and MeIQ, respectively) or women ($P_{interaction} =$ 0.84 and 0.54 for HCA and MeIQ, respectively).

Discussion

In this colonoscopy-based study, we observed an increased risk of colorectal adenoma associated with the intake of processed meat, MeIQ, and total HCA in women but not in men. No clear association between *NAT2* acetylation genotype and colorectal adenoma risk was found in either men or women, nor was any evidence seen of effect modification by *NAT2* acetylation genotype on the association between HCA estimates and colorectal adenoma risk. To our knowledge, this is the first study to investigate the association of HCA intake with colorectal adenoma risk in an Asian population.

MeIQ concentrations were not detected in any meat sample evaluated by Sinha and colleagues (7), and these are accordingly not evaluated in studies using the CHARRED database to estimate HCA measures. In contrast, MeIQ concentrations in cooked fish were measurable in our database (28), and the large intake of fish items in our population allowed inclusion of MeIQ concentration estimates. Our finding of an increased risk of colorectal adenoma with MeIQ in women is partly supportive of a study of colorectal cancer in Sweden (24), albeit that its MeIQ estimates were lower (<1 ng/d) than the present study. Although MeIQ is less abundant in food than PhIP, it is a more potent mutagen (11), which could partly explain the positive association of MeIQ with colorectal adenoma risk. However, the associations observed in the present study were limited to women only. The reason for the discrepant findings in men and women is unknown. The amount of intake of fish and meat items that most strongly contributed to MeIQ intake was similar in men and women. Of importance, however, a lower proportion of men (56%) than women (62%) reported grilling as their preferred method of cooking fish. For meat items, 45% of women preferred medium done meat whereas 46% preferred nearly well-done or well-done meat. In contrast, 57% of men preferred medium doneness and only 28% preferred nearly well-done or well-done meat. Compared with women, therefore, the men in our study might have been homogenous with regard to their preference for cooking method/doneness, which in turn might have impacted the ability to demonstrate a significant

Table 4. Multivariate-adjusted OR and 95% CI of colorectal adenoma according to *NAT2* acetylation genotype

	Men		Women	
***NAT2* acetylation genotype**	**Case/controls**	**OR (95% CI)[a]**	**Case/controls**	**OR (95% CI)[a]**
Slow	40/34	1.00 (ref.)	19/14	1.00 (ref.)
Intermediate	187/169	1.00 (0.59–1.68)	90/99	0.64 (0.29–1.41)
Rapid	220/207	0.91 (0.54–1.52)	108/103	0.69 (0.32–1.52)
P_{trend}		0.54		0.69
Rapid vs. slow/intermediate		0.91 (0.69–1.20)		1.01 (0.68–1.51)

[a]Adjusted for age, screening period, smoking, alcohol consumption, body mass index, physical activity, family history of colorectal cancer, and NSAID use. Further adjusted for age at menarche, menopausal status, and current use of hormones in women.

Table 5. Association between total HCA and MeIQ estimate and colorectal adenoma risk according to *NAT2* acetylation genotype

Quartile (Q)	*NAT2* acetylation genotype							
	Men				Women			
	Slow/intermediate		Rapid		Slow/intermediate		Rapid	
	N	OR (95% CI)[a]	N	OR (95% CI)[a]	N	OR (95% CI)[a]	N	OR (95% CI)[a]
HCA								
Q1	41/45	1.00 (ref.)	56/58	1.07 (0.59–1.91)	18/28	1.00 (ref.)	20/27	0.93 (0.38–2.23)
Q2	78/47	1.97 (1.10–3.54)	56/54	1.11 (0.62–2.00)	20/26	1.00 (0.41–2.44)	24/24	1.18 (0.49–2.82)
Q3	44/61	0.82 (0.45–1.50)	62/38	1.77 (0.96–3.26)	35/32	1.22 (0.54–2.77)	31/24	1.43 (0.61–3.34)
Q4	64/50	1.36 (0.75–2.44)	46/57	0.83 (0.46–1.52)	36/27	1.81 (0.77–4.23)	33/28	1.53 (0.67–3.50)
$P_{interaction}$		0.81				0.84		
MeIQ								
Q1	45/45	1.00 (ref.)	56/55	0.98 (0.55–1.76)	15/27	1.00 (ref.)	18/26	0.94 (0.37–2.39)
Q2	65/49	1.39 (0.78–2.50)	64/57	1.11 (0.63–1.96)	29/29	1.49 (0.62–3.55)	22/22	1.35 (0.54–3.38)
Q3	55/62	0.92 (0.52–1.63)	49/37	1.28 (0.69–2.39)	31/26	1.75 (0.72–4.23)	28/29	1.47 (0.63–3.47)
Q4	62/47	1.21 (0.67–2.17)	51/58	0.79 (0.44–1.43)	34/31	1.68 (0.71–3.93)	40/26	2.26 (0.96–5.30)
$P_{interaction}$		0.60				0.54		

Abbreviation: N, number of cases/controls.

[a]Adjusted for age, screening period, smoking, alcohol consumption, body mass index, physical activity, family history of colorectal cancer, and NSAID use. Further adjusted for age at menarche, menopausal status, and current use of hormones in women.

difference in risk estimates. Of further note, the correlation coefficient for validity of MeIQ intake was lower in men than in women, and this variability in HCA measurement could have contributed to the attenuation of risk in men.

In our study, the association of PhIP and MeIQx with colorectal adenoma risk was not clear; OR in the highest quartile suggested an increased risk but lacked statistical significance. Findings for individual HCA in the previous studies were mixed, however, even within the same study. Among examples, Ferrucci and colleagues (34) reported that MeIQx, but not PhIP or DiMeIQx, was associated with an increased risk of colon adenoma in women, whereas in their case–control study in a multiethnic population in Hawaii, Wang and colleagues (35) found that only DiMeIQx was positively associated with colorectal adenoma risk. In their prospective study, Rohrmann and colleagues observed that the positive association between HCA and colorectal adenoma risk remained significant only with the PhIP intake estimates, whereas the relation with the other 2 HCA was lost after multivariate adjustment (18). The estimated intake of PhIP and MeIQx in the present study was lower than in the other studies; for example, our respective median PhIP and MeIQx estimates were 18 and 4 ng/d, compared with 38 and 12 ng/d in one study (17) and 55 and 19 ng/d in another (16). Also, our validation study showed that MeIQ was estimated more accurately than the other 2 HCA (32, 33); such measurement error, compounded by differences in the carcinogenic potential of individual HCA compounds, might have contributed to both our less significant association with PhIP and MeIQx in the present study and inconsistencies in findings among the previous studies.

A recent meta-analysis of 26 studies (36), mostly European and American, reported that red meat and processed meat consumption was associated with a significantly increased risk of colorectal adenoma, with a summary relative risk of 1.27 (95% CI, 1.16–1.40) and 1.29 (95% CI, 1.10–1.53) per 100 and 50 g/d increases in the intake of red and processed meat, respectively. However, only 3 studies in this meta-analysis referred to red meat intake in Asians (37–39). Of these, a study of 59 case–control pairs in Malaysia (37) reported a significant increase in risk with red meat intake (OR for $\geq$3 times/wk compared with <3 times/wk was 2.51; 95% CI, 1.00–6.28), whereas the other 2 studies were unclear (38, 39). The present study finding showed no clear effect of red meat in colorectal adenoma and is accordingly consistent with previous reports of colorectal adenoma (39) or cancer (40) in Japanese populations. Red meat intake in our study was similar to that in the other Japanese studies (39, 40) but lower than the average intake in the Western populations and might therefore have been insufficient (40) to produce a substantive difference in the risk estimates of colorectal adenoma.

Processed meat, treated with nitrates and nitrites, might be an exogenous source of *N*-nitroso compounds (NOC) exposure in humans. Furthermore, processed meat also contains all the necessary precursors (nitrite, amines, and amides) for endogenous formation of NOC (41). NOCs have been found to be potent carcinogens in experimental studies and might explain the increased risk of colorectal cancer by processed meat consumption observed in epidemiologic studies (13, 25, 41). In Japan, the Ministry of Agriculture, Forestry and Fisheries has approved the use of nitrite, in the form of sodium nitrite, for coloring purposes in meat products, whale meat bacon, fish sausage, fish ham, salmon roe, roe, and cod roe (42), and in most cases, ascorbic acid is also used together with nitrite. However, the processed meat in our study consisted of ham, sausage, and bacon and did not include processed fish. We do not have information on nitrite use for salted and dried fish products, canned tuna, and fish paste products. However, when we analyzed the data after adding these fish products to the processed meat group, we saw no association in men, whereas the positive association observed in the original processed meat group in women was lost (data not shown). The null association of fish intake with colorectal adenoma was also unchanged when we excluded salted and dried fish products, canned tuna, and fish paste products from the original fish group. Although the present study suggested an increased risk with processed meat intake in women, as with red meat, the intake amount of processed meat was very small compared with the other studies (25, 43). The finding might therefore be the result of chance and should be interpreted cautiously.

Among other findings, we observed no association between *NAT2* acetylation genotype and colorectal adenoma risk in the present study. With an estimated OR of 0.94 (95% CI, 0.86–1.03), a recent meta-analysis that included 3,683 adenoma cases and 5,109 controls also failed to find any evidence of an increased risk of colorectal adenoma in individuals with *NAT2* rapid genotype

(44). It has been suggested that these common genetic variants may have only a small direct effect on risk and might not manifest any risk when exposure to the carcinogen is low and biologically insufficient (12). A few other studies found a statistically significant increased risk in rapid *NAT2* acetylators with high HCA intake (22) or high processed meat intake (45) only or among individuals with the rapid phenotype for *NAT2* and *CYP1A2* and a preference for well-done or very well-done red meat (14). However, other studies failed to find such an association (46, 47), and the *P* value for interaction between HCA intake and *NAT2* phenotype in these (14, 22, 45) and other studies (46, 47) was not significant.

Our study has several strengths. All participants underwent total colonoscopy, which likely reduced the possibility of misclassification of case and control status. Dietary and lifestyle factors were ascertained before colonoscopy, likely minimizing the possibility of recall bias. Dietary habits were assessed using a validated FFQ. HCA intake was estimated using a database specifically developed for our FFQ and was validated against PhIP content in hair. Furthermore, our focus on adenoma eliminated the possibility of survival bias.

Several limitations of the study also warrant mention. These include its cross-sectional design and retrospective assessment of dietary and other lifestyle factors. Furthermore, dietary intake was assessed for the recent past, which might not have represented the period in which the colorectal adenoma developed. Although our questionnaires were previously validated, the possibility of measurement error remains, as shown by the modest correlation coefficient for validity. Although we adjusted for known or potential confounding factors in multivariable models, residual or unmeasured confounding remains possible. Furthermore, we lacked information on other carcinogenic compounds, such as PAH and NOCs exposure. Nevertheless, the carcinogenic potential of different HCAs or other carcinogenic compounds might be additive or possibly synergistic, in which case the risk estimates would likely be stronger (12). We evaluated a single metabolic enzyme only (NAT2). As several phase I and other phase II enzymes are also involved in the metabolism of HCA, we consider that accounting for all these enzymes would likely accurately capture the true risk and should be considered in future studies. Furthermore, the *NAT2* rapid and slow acetylation genotypes were inferred by genotyping only 3 SNPs, which might have caused some rapid acetylators to be misclassified as slow. Nevertheless these 3 SNPs were shown to infer acetylation phenotypes with 100% accuracy among a Japanese population (48), and any such misclassification is likely to be small.

In summary, we found a significant positive association between higher MeIQ intake and colorectal adenoma risk in women but not in men. Our findings in women suggest a role of MeIQ or HCA in colorectal carcinogenesis. These findings should be verified in larger studies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Iwasaki, T. Yamaji, H. Sakamoto, T. Yoshida, S. Tsugane
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Writing, review, and/or revision of the manuscript: S. Budhathoki, M. Iwasaki, T. Yamaji, S. Sasazuki, R. Takachi, T. Yoshida, S. Tsugane
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): R. Takachi, H. Sakamoto, T. Yoshida
Study supervision: M. Iwasaki, S. Tsugane

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