Meat Consumption, Genetic Susceptibility, and Colon Cancer Risk: A United States Multicenter Case-Control Study[1]

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Abstract

Meat consumption may especially increase risk of colon cancer when the meat is prepared at high temperatures and consumed by subjects with an inherited susceptibility to well-done meat. In this United States case-control study, the association between meat consumption, genetic susceptibility, and colon cancer risk was studied. Meat consumption data were available from a detailed diet history questionnaire and from questions about methods of preparation. Molecular variants in the carcinogen-metabolizing genes *NAT2* and *GSTM1* were determined in DNA extracted from WBCs. A total of 1542 cases and 1860 population-based controls were included in these analyses.

The amount of red and white meat consumed was not associated with overall colon cancer risk. Processed meat consumption was weakly positively associated with colon cancer risk in men only (odds ratio for highest *versus* lowest quintile of intake = 1.4, 95% confidence interval = 1.0–1.9). The frequency of fried, broiled, baked, or barbecued meat, use of drippings, and doneness of meat were not significantly associated with risk. The Mutagen Index, as an estimate for exposure to mutagenic or carcinogenic substances, was slightly positively associated with colon cancer risk in men (odds ratio = 1.3, 95% confidence interval = 1.0–1.7). No significant associations with colon cancer risk were observed for different *NAT2* and *GSTM1* gene variants. The observed associations with processed meat and the Mutagen Index were strongest for those with the intermediate or rapid NAT2 acetylator phenotype. Associations were not markedly influenced by lack of the *GSTM1* gene.

This study provides little support for an association between meat consumption and colon cancer risk but does provide some, albeit not strong, evidence for a modifying effect of molecular variants of the *NAT2* gene.

Introduction

In contrast to vegetarian diets or other diets high in plant foods, a diet rich in meat and meat products has been associated with an excess in colon cancer risk in several epidemiological studies (1). However, results have not been consistent: the consumption of pork, beef, lamb, or red meat (as one food group) significantly increased colon cancer risk ~2–3-fold in two United States cohort studies (2, 3) and several case-control studies (4–9), although other prospective (10–12) and retrospective studies (13–25) have not reported a significant association. For poultry and fish consumption, results have been more consistent: most studies observe a null or an inverse association (2, 3, 6, 9, 11, 12, 14, 16, 19–23, 25, 26). Only a few studies have evaluated the risk with processed meat: nearly 2-fold increases in risk have been observed (5, 12, 26).

Besides methodological differences between studies, inconsistencies between study results might be explained by the different preparation methods habitually used in different populations. Preparation methods influence the content of mutagenic and carcinogenic compounds in meat and meat products; mutagenic activity has been demonstrated in red meat and chicken cooked at relatively high temperatures and in drippings often used for the preparation of gravy (27). Mutagens have been reported in urine and feces of those who consumed fried meats (28). Regular consumption of well-done or fried meats have been associated with 2–3-fold increases of colon cancer risk in some (8, 29, 30) but not other studies (31, 32).

Differences between studies may also be explained by the genetic heterogeneity of the study populations. Potential carcinogens in meat prepared at high temperatures, *e.g.*, heterocyclic amines and polycyclic aromatic hydrocarbons, are metabolized by enzymes, such as the *N*-acetyltransferases (*e.g.*, NAT2) and GSTs[4] (*e.g.*, GST μ), the activities of which are genetically variable across the population. Molecular variants of the *NAT2* and *GSTM1* genes are common and may be associated with altered colon cancer risk, although the magnitude of this elevation appears to be small (OR $<$ 2; Refs. 33–36). When combined with exposure to environmental carcinogens, however, the impact of these variants on risk may be larger: *e.g.*, rapid acetylators (*i.e.*, those with the wild-type *NAT2* allele) were observed to be at a 6-fold increased risk of colorectal cancer among those who frequently consume fried

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[4] The abbreviations used are: GST, glutathione *S*-transferase; OR, odds ratio; CI, confidence interval; BMI, body mass index.

meat in a case-control study in England, including 174 cases and 174 controls (37). The prospective Physician's Health Study also found this stronger increased risk with meat consumption among NAT2 rapid acetylators, especially among those men 60 years and older (36).

In this case-control study, including 1542 colon cancer cases and 1860 population-based controls, we evaluated whether meat consumption was associated with risk and whether this risk was modified by molecular variants of the *NAT2* and *GSTM1* genes.

Materials and Methods

These analyses were conducted as part of the Diet, Activity and Reproduction Study of Colon Cancer. Participants were recruited from the Kaiser Permanente Medical Care Program of Northern California; an eight-county area in Utah (Davis, Morgan, Salt Lake, Summit, Tooele, Utah, Wasatch, and Weber Counties); and the metropolitan Twin Cities area in Minnesota (Anoka, Carver, Dakota, Hennepin, Ramsey, Scott, and Washington Counties). Within these defined areas, all eligible cases were identified.

To be eligible, participants had to be between 30 and 79 years old; study area residents; able to speak English; mentally and physically able to participate; and without history of colorectal cancer, familial adenomatous polyposis, ulcerative colitis, or Crohn's disease. Cases and controls were interviewed between February 1992 and April 1995. The ethnic distribution of the study population was 91% white, 4.5% Hispanic, and 4.5% African-American.

Cases. Cases had a first primary colon carcinoma (International Classification of Diseases for Oncology Edition 2 codes 18.0 and 18.2–18.9) diagnosed between October 1, 1991, and September 30, 1994. Because epidemiological studies suggest different risk factors for colon and rectal cancers, cases with tumors of the appendix, rectosigmoid junction, or rectum were not eligible. Complete case ascertainment was verified through local tumor registries. Response and cooperation rates have been described previously (38). In brief, 64.5% of those eligible were interviewed. Interviews for the large majority were completed within 4 months of diagnosis. The median time from diagnosis to interview for all subjects was 131 days.

Controls. Controls were frequency-matched to cases by sex and 5-year age group. Methods to recruit controls have been outlined (39). In short, Kaiser Permanente Medical Care Program controls were selected from membership lists. In Utah, controls under 65 were identified using random-digit dialing and the state driver's license and identification list, whereas those 65 and older were selected using Health Care Financing Administration data. In Minnesota, controls were randomly selected from the state driver's license and identification list. For the entire study, 63% of those contacted were interviewed. No significant age and sex differences were observed in response or cooperation rates (39).

Dietary and Lifestyle Data Collection. Data were collected using a detailed interviewer-administered questionnaire (40). Participants were asked to recall the 12-month period 2 years prior to the reference date (the date of diagnosis for cases and date of selection for controls). Dietary intake was ascertained using an adaptation of the dietary history interview-based questionnaire that was developed and validated for the study on Coronary Artery Risk Development in Young Adults (41, 42). With this instrument, study participants had the option of reporting on food items that were eaten at least once per month and, in the case of meat items, at least once per year; over 800 separate food items were listed in the questionnaire. The frequency with which foods were eaten, fat used in the preparation of foods, and information on foods eaten as additions were obtained. Nasco three-dimensional food models, plastic cups, and spoons were used to help participants identify usual serving sizes. Cue cards were used to help in the identification of individual food items from broader food categories. Nutrient information was calculated using the Nutrition Coordinating Center Nutrient Database, Version 19 (43).

Specific questions on the preparation of red meat, poultry and fish were used, including those on the preferred degree of cooking ("doneness") of red meat and poultry (rare, medium-rare, medium-well done, and well done); the frequency of cooking by frying, broiling, baking, or barbecuing of red meat, poultry, and fish; and the frequency of the use of drippings of red meat, poultry, and fish, either on other foods or in gravy. We estimated cooking temperature and, therefore, potential exposure to mutagens, by calculating a mutagen index. The index is calculated as the frequency of red meat, poultry, and fish consumption prepared by frying, broiling, baking, or barbecuing plus the use of drippings from red meat, poultry, or fish, multiplied by the preferred doneness of the red meat, poultry, and fish (1 = rare, 2 = medium-rare, 3 = medium-well done, 4 = well done) and the microwave factor (1 = microwave never used or used for thawing, 0.75 = sometimes used, 0.5 = often used, 0.25 = always used). A high index reflects higher intake of potentially mutagenic compounds.

The interview also included questions on demographics, reproductive history, long-term physical activity, medical history, and family history of polyps, colorectal cancer, and other cancers. Height was measured at the time of the interview, and weight was self-reported for the referent period. BMI for the referent period was calculated as weight/(height)$^{1.5}$ for women (44) and weight/(height)^2 for men. The physical activity questionnaire was adapted from one developed and validated for Coronary Artery Risk Development in Young Adults (45). The study methods were approved by the Ethical Committees and Internal Review Boards of the participating study centers.

Genotyping Assays. Genomic DNA was extracted from peripheral WBCs using the PureGene DNA isolation kit (Gentra Systems Inc., Minneapolis, MN) for samples obtained from Minnesota and Kaiser. In Utah, DNA was obtained from immortalized cell lines.

NAT2 genotyping was performed using an oligonucleotide ligation assay as described previously (46). This assay allows the use of 96-well plates and a robotic workstation. A single PCR with an input of 50–100 ng of genomic DNA provides sufficient amplified NAT2 fragments to analyze the five missense mutations. Briefly, primers 5′-GGAACAAATTGGACTTGG-3′ and 5′-TCTAGCATGAATCACTCTGC-3′ (47) were used to amplify the NAT2 coding region from 100 ng of genomic DNA in 50-μl reactions containing 10 mM Tris (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin (Perkin-Elmer Corp., Foster City, CA), 50 μg/ml BSA, 0.2 μM primers, 0.2 mM dNTPs, 1 unit of Amplitaq DNA polymerase (Perkin-Elmer Corp.). The cycling conditions were: 4 min at 94°C; 40 cycles at 94°C for 30 s, 57°C for 45 s, and 72°C for 90 s; and a final extension at 72°C for 5 min (46). For the ligation, the PCR was diluted with 80 μl of 0.1% Triton X-100. The 20-μl ligation reactions consisted of 10 μl of diluted PCR product, 20 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 12.5 mM KCl, 1 mM DTT, 1 mM NAD, 0.1% Triton X-100, 8 fmol/μl biotinylated wild-type or mutant primer, 8 fmol/μl digoxigenin-tailed common primer [for primer sequences see Bigler *et al.* (46)], and 0.015 units of

Table 1 Characteristics of the study population according to selected nutrients and meat consumption[a]

Dietary variable	Men			Women		
	Cases (n = 868)	Controls (n = (989)	P	Cases (n = 674)	Controls (n = 871)	P
Nutrients						
Total energy (kcal)	2792 (1186)	2646 (1162)	0.02	2051 (869)	1972 (832)	0.07
Total fat						
g/day	104 (55)	98 (55)	0.01	75 (42)	70 (39)	0.02
g/1000 kcal	37 (8)	36 (8)	0.02	35 (8)	35 (8)	0.03
Total protein						
g/day	104 (44)	101 (46)	0.13	79 (33)	78 (33)	0.34
g/1000 kcal	38 (7)	39 (7)	0.16	39 (7)	40 (7)	0.06
Animal protein (g/day)	69 (34)	66 (35)	0.04	51 (24)	50 (24)	0.33
Calcium (mg/day)	1196 (636)	1220 (649)	0.42	962 (539)	976 (526)	0.59
Cholesterol (mg/day)	360 (230)	324 (216)	<0.01	243 (149)	224 (130)	<0.01
Foods in servings per week						
Red meat	6.4 (5.2)	5.9 (5.0)	0.02	4.3 (3.5)	4.1 (3.5)	0.21
Poultry	1.8 (1.7)	1.9 (2.3)	0.75	1.8 (1.5)	1.7 (1.5)	0.54
Processed meat	2.4 (2.3)	2.0 (2.1)	<0.01	1.2 (1.4)	1.1 (1.2)	0.09
Fish	2.0 (2.6)	2.0 (2.4)	0.60	1.5 (1.6)	1.5 (1.5)	0.98

[a]Values are means (SD).

thermostable ligase (Epicentre Technologies, Madison, WI). The cycling conditions for the ligation for all of the mutations were: 15 cycles of 93°C for 30 s and 58°C for 2 min. The reaction was stopped with 10 μl of a buffer containing 0.1 M EDTA (pH 8.0) and 0.1% Triton X-100.

The ligation reactions were transferred into streptavidin-coated 96-well plates. After incubation for 60 min at room temperature, the plates were washed twice with 10 mM NaOH-0.05% Tween 20, followed by two washes with 200 μl of 100 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 0.05% Tween. The plates were then incubated with 40 μl of a 1000-fold dilution of antidigoxigenin Fab fragment-alkaline phosphatase conjugate (0.75 units/μl; Boehringer Mannheim, Indianapolis, IN) for 30 min at room temperature. After four washes with 100 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 0.05% Tween 20, the Life Technologies, Inc., ELISA amplification system was applied for the color reaction according to the manufacturer's recommendations. The absorbance at 495 nm was recorded using a SpectraMax 250 plate reader (Molecular Devices, Sunnyvale, CA).

We limited our analysis to the *NAT2* missense mutations G191A, T341C, G590A, A803G, and G857A. The two other known missense mutations, A434C and A845C, are not included because of their low frequency of occurrence (48–50), and the three silent mutations at nucleotide positions 282, 481, and 759 are not included because they do not appear to influence enzyme activity (48, 49, 51, 52). *NAT2* genotyping was conducted for 1624 cases and 1943 controls.

The *GSTM1* null genotype was detected using the technique described by Zhong and colleagues (53). *GSTM1* genotyping was conducted for 1567 cases and 1889 controls.

Data Analysis. Data analysis included those with complete environmental and genotyping data, *i.e.*, 1542 cases and 1860 controls. Of those interviewed, 90 cases and 66 controls were excluded from the analysis either because they were identified as ineligible at interview or because of missing data or data considered to be of poor quality by the interviewer. The blood samples collected allowed DNA extraction and subsequent amplification for 77% of cases and controls interviewed. Those with genotype data were slightly older (64.9 *versus* 63.1 years) than those without genotype data and drank more alcohol (14.6 g/day *versus* 11.7 g/day). No other differences in dietary and other lifestyle factors were observed.

Nutrients were analyzed using the density method (38). In general, categorization of the variables of interest was based upon the distribution of the control population. ORs and approximate 95% CIs were calculated by unconditional maximum likelihood estimation using BMDP software.

All analyses are adjusted for age at diagnosis (cases) or selection (controls), BMI, lifetime physical activity, total energy intake, usual number of cigarettes smoked per day, and intake of dietary fiber. Cholesterol, fat, and protein were not included in the model to avoid overcontrolling. Other lifestyle factors, such as alcohol consumption, did not influence risk estimates significantly. Subjects with unknown values for any potential confounding variable were excluded.

Analyses were stratified by sex, age at diagnosis (using the median age of the controls, 67 years), number of cigarettes smoked per day (<20 *versus* ≥20 per day), and subsite of the colon. For subsite comparisons, proximal colon tumors are defined as tumors in the cecum, ascending colon, hepatic flexure, and transverse colon. Distal tumors were defined as those from the splenic flexure to the sigmoid.

Results

Table 1 presents the characteristics of the 1542 cases and 1860 controls included in these analyses, according to the intake of meat-related nutrients and meat consumption. [We have reported previously on the role of a variety of other risk factors, including physical activity and obesity (38), fat (54), plant foods (55), tobacco (56), and hormone replacement therapy (57).] Energy intake, the percentage of energy consumed as fat, and the intake of total fat and cholesterol were significantly higher among colon cancer cases of both sexes. Animal protein intake was higher among male cases than controls. Red meat and processed meat were more frequently consumed by cases than controls, but this difference was statistically significant only for men. For the consumption of fish and poultry, no differences between cases and controls were observed.

Adjustment for confounding variables resulted in marginal changes of the ORs; only the full models are presented in

Table 2 Amount of meat consumed per week and colon cancer risk: ORs and 95% CIs[a]

Food group	Men		Women	
	Servings/week[b]	OR (95% CI)	Servings/week[b]	OR (95% CI)
Red meat[c]	≤2.2	1.0	≤1.5	1.0
	2.3–3.7	0.8 (0.6–1.0)	1.6–2.5	1.1 (0.8–1.5)
	3.8–5.6	1.1 (0.8–1.0)	2.6–4.0	1.3 (0.9–1.8)
	5.7–8.8	1.0 (0.7–1.4)	4.1–6.2	1.3 (0.9–1.8)
	>8.8	0.9 (0.7–1.3)	>6.2	1.0 (0.7–1.5)
Poultry[d]	≤0.5	1.0	≤0.5	1.0
	0.6–1.0	0.9 (0.7–1.2)	0.6–1.1	1.1 (0.8–1.5)
	1.1–1.6	1.0 (0.8–1.4)	1.2–1.7	0.9 (0.6–1.3)
	1.7–2.8	1.0 (0.7–1.3)	1.8–2.7	1.0 (0.7–1.3)
	>2.8	1.0 (0.8–1.4)	>2.7	1.1 (0.8–1.5)
Processed meat[e]	≤0.5	1.0	≤0.2	1.0
	0.6–1.0	1.1 (0.8–1.6)	0.3–0.5	1.3 (1.0–1.9)
	1.1–1.8	1.2 (0.9–1.8)	0.6–0.9	1.2 (0.9–1.7)
	1.9–3.1	1.3 (1.0–1.8)	1.0–1.7	1.3 (0.9–1.8)
	>3.1	1.4 (1.0–1.9)	>1.7	1.1 (0.8–1.6)
Fish[f]	≤0.4	1.0	≤0.3	1.0
	0.5–0.9	1.2 (0.9–1.7)	0.4–0.8	0.9 (0.7–1.3)
	1.0–1.7	1.1 (0.8–1.5)	0.9–1.4	0.8 (0.6–1.2)
	1.8–3.1	1.4 (1.0–1.8)	1.5–2.5	1.1 (0.8–1.5)
	>3.1	1.1 (0.8–1.5)	>2.5	0.8 (0.6–1.2)

[a] Adjusted for age, calories, BMI, long-term activity, dietary fiber, and usual no. of cigarettes smoked.
[b] Amount consumed, standardized to a 3-ounce serving.
[c] Red meat includes round beef, hamburger, ground beef casseroles, hamburger helper, pot roast, steaks, and ham.
[d] Poultry includes chicken, turkey, Cornish game hens, duck, goose, chicken and turkey salad, and fast-food poultry.
[e] Processed meat includes bacon, sausages, and cold cuts.
[f] Fish includes fresh, frozen, smoked, canned, shellfish, and fast-food fish.

Tables 2–6. For men, processed meat consumption was significantly positively associated with risk; for fish, a statistically nonsignificant increase in colon cancer risk was observed (Table 2). For women, no marked associations with red or white meat consumption were observed. Results did not change significantly following stratification on age, BMI (above and below median level), number of cigarettes smoked per day, or colonic subsite (data not shown).

The consumption of organ meats in this population was infrequent and was not associated with risk (OR users *versus* nonusers = 1.10; 95% CI = 0.93–1.32). No marked association was observed with the ratio of red meat to all flesh foods (data not shown).

Table 3 shows data on variables associated with exposure to potentially mutagenic and carcinogenic compounds. The frequencies of red meat prepared at high temperatures, *i.e.*, fried, broiled, baked, or barbecued red meat, and the use of red meat drippings were not significantly associated with colon cancer risk. Although there seems to be a slight increase in risk with doneness of red meat, the trend was not statistically significant. No significant association was observed for white meat prepared at high temperatures, although the white meat mutagen index was slightly associated with risk in men (Table 3). The overall mutagen index for red meat and white meat together was also significantly positively associated with colon cancer risk in men (Table 3). Further stratification revealed stronger associations with the mutagen index for women at older ages as compared to women younger than 67 years (OR for highest *versus* lowest mutagen index women 67 years and older = 1.3, 95% CI = 0.9–1.9; OR for women younger than 67 years = 0.9, 95% CI = 0.6–1.3). Moreover, the strongest associations with the mutagen index were observed among both sexes for those with distal colon tumors (OR for men = 1.6, 95% CI = 1.2–2.3; OR for women = 1.2, 95% CI = 0.9–1.7).

About 40% of the study population carried the wild-type *NAT2*4* allele and were considered to be intermediate or rapid acetylators. Although no association was observed for rapid acetylator status, the intermediate acetylator NAT2 phenotype slightly increased colon cancer risk in women (Table 4). For men, no significant association for intermediate or rapid phenotype was observed (Table 4). Specific *NAT2* genotypes for cases and controls and for men and women separately are included in the Appendix. No significant differences in colon cancer risk were observed across specific *NAT2* genotypes (data not shown). A homozygous deletion of the *GSTM1* gene was observed for ~50% of the study population; it was not significantly associated with colon cancer risk in either sex (Table 4).

In general, associations with red meat consumption and preparation methods appeared somewhat, although not statistically significantly, stronger for those with the intermediate or rapid NAT2 phenotype (Table 5). This difference between phenotypes was especially important for the consumption of processed meat and the red meat mutagen index (Table 5). In contrast, and unexpectedly, white meat appeared to be more strongly associated with risk among slow acetylators. The overall mutagen index for red and white meat together was significantly positively associated with colon cancer risk only among intermediate and rapid acetylators (Table 5).

Stratification did not reveal marked differences between those younger than 67 years and those 67 years and older (data not shown). Stratification on colon subsites showed strongest differences among NAT2 phenotypes for those with distal tumors, especially for those with a high red meat mutagen index as compared to those with a low index (OR for slow acetylators = 1.0 and OR for intermediate/rapid acetylators = 1.4, 95% CI = 1.0–2.0); this was most apparent among women (OR for slow acetylators = 0.9 and OR for intermediate/rapid acetylators = 1.6, 95% CI = 1.0–2.6).

For the *GSTM1* genotypes, in contrast to what might be expected, the strongest positive associations were observed for those in which the *GSTM1* gene was present (Table 6). For red meat, no significant differences were observed. Significant positive associations were found for the frequency of white meat consumption prepared at high temperatures and for the white meat mutagen index, but only for those with the *GSTM1* gene present (ORs for highest *versus* lowest category = 1.4; Table 6). The overall mutagen index was positively associated with risk among those both with and without an intact *GSTM1* gene (Table 6).

No significant differences were observed stratifying for age or between men and women with different *GSTM1* genotypes. As with the NAT2 findings, differences between *GSTM1* genotypes appeared marginally stronger for the distal part of the colon (data not shown).

Discussion

This case-control study found little association between meat consumption and colon cancer risk. There is some suggestion that meat consumption, especially processed meat, contributes to risk especially among those with an intermediate or rapid NAT2 acetylator phenotype. There is very modest support for the idea that the method of preparation may be more important than the absolute amount consumed, mainly among intermediate or rapid acetylators. A homozygous deletion of the *GSTM1* gene was not found to be associated with colon cancer risk, nor was it significantly associated with modifications of the meat consumption and preparation risk estimates. Associations ap-

Table 3 Meat preparation, drippings, Mutagen Index, and colon cancer risk: ORs and 95% CIs[a]

Variable	Men		Women	
	Frequency per year	OR (95% CI)	Frequency per year	OR (95% CI)
Fried, broiled, baked, or barbecued red meat	≤52	1.0	≤48	1.0
	53–104	1.0 (0.8–1.3)	49–52	1.0 (0.7–1.4)
	105–156	1.2 (1.0–1.6)	53–104	1.2 (0.9–1.6)
	157–208	1.0 (0.7–1.3)	105–156	1.1 (0.8–1.5)
	>208	1.3 (0.9–1.7)	>156	0.9 (0.6–1.3)
Doneness of red meat	Rare/medium rare	1.0	Rare/medium rare	1.0
	Medium well done	1.2 (0.9–1.5)	Medium-well done	1.1 (0.8–1.4)
	Well done	1.2 (0.9–1.5)	Well done	1.2 (0.9–1.5)
Use of red meat drippings	Never	1.0	Never	1.0
	1–6	0.8 (0.5–1.1)	1–4	0.8 (0.5–1.2)
	7–24	0.8 (0.6–1.1)	5–24	0.9 (0.7–1.2)
	25–76	0.8 (0.6–1.1)	25–64	1.0 (0.7–1.3)
	>76	0.9 (0.7–1.2)	>64	1.0 (0.8–1.4)
Red meat Mutagen Index	Low	1.0	Low	1.0
	2	0.8 (0.7–1.1)	2	0.9 (0.7–1.2)
	3	1.1 (0.9–1.4)	3	0.9 (0.7–1.2)
	High	1.0 (0.8–1.3)	High	1.1 (0.8–1.1)
Fried, broiled, baked, or barbecued white meat	≤36	1.0	≤52	1.0
	37–52	1.2 (0.9–1.5)	53–60	0.9 (0.3–2.7)
	53–104	1.1 (0.9–1.5)	61–104	1.0 (0.7–1.3)
	105–156	1.3 (0.9–1.8)	105–156	0.9 (0.7–1.2)
	>156	1.1 (0.8–1.6)	>156	1.2 (0.9–1.6)
White meat Mutagen Index	Low	1.0	Low	1.0
	2	1.1 (0.8–1.3)	2	1.1 (0.9–1.5)
	3	1.1 (0.9–1.4)	3	1.0 (0.8–1.3)
	High	1.3 (1.0–1.6)	High	1.0 (0.8–1.4)
Overall Mutagen Index	Low	1.0	Low	1.0
	2	1.2 (0.9–1.5)	2	1.0 (0.8–1.3)
	3	1.1 (0.8–1.4)	3	1.0 (0.8–1.4)
	High	1.3 (1.0–1.7)	High	1.1 (0.8–1.4)

[a] Adjusted for age, BMI, calories, dietary fiber, lifetime physical activity, and usual no. of cigarettes smoked.

Table 4 NAT2, GSTM1, and colon cancer risk: ORs and 95% CIs[a]

Imputed phenotype	Men			Women		
	No. (%)		OR (95% CI)	No. (%)		OR (95% CI)
	Cases	Controls		Cases	Controls	
NAT1						
Slow	536 (58.8)	603 (58.2)	1.0	394 (55.3)	553 (59.7)	1.0
Intermediate	323 (35.4)	361 (34.8)	1.0 (0.8–1.2)	281 (39.5)	322 (34.7)	1.2 (1.0–1.5)
Fast	53 (5.8)	72 (7.0)	0.9 (0.6–1.2)	37 (5.2)	52 (5.6)	1.0 (0.7–1.6)
Intermediate or fast	376 (41.2)	433 (41.8)	1.0 (0.8–1.2)	318 (44.7)	374 (40.3)	1.2 (1.0–1.5)
GSTM1						
Present	436 (49.3)	499 (49.1)	1.0	323 (47.9)	386 (44.2)	1.0
Null	448 (50.7)	518 (50.9)	1.0 (0.8–1.2)	360 (52.1)	486 (55.8)	0.9 (0.7–1.0)

[a] Adjusted for age, BMI, calories, dietary fiber, lifetime physical activity, and usual no. of cigarettes smoked.

peared marginally stronger for those with tumors in the distal part of the colon. In general, even associations that were statistically significant were weak.

Although this is one of the largest case-control studies to date, biases associated with a retrospective design are always an issue. As we have shown previously, the control population is not significantly different from the population at large (39), and the educational level is similar among cases and controls. Cases and controls were interviewed in a standardized manner, decreasing the likelihood of differential misclassification.

For red meat, the findings are consistent with those of other studies in which no strong overall associations have been observed (10–20, 23, 24). The increased risk of colon cancer with an increased intake of processed meat has been observed in a number of other studies (5, 12, 26). However, in our study, a marked association was observed only among men.

Although the absolute amount of red meat consumed was not associated with colon cancer risk, the doneness of meat was associated with elevated risk. This modest increase in risk is consistent with the findings of others (18, 31). The concentration of the heterocyclic amine 2-amino-1-methyl-6-phenylimidazo-[4,5-β]pyridine in red meat and chicken has been shown to be

Table 5 Meat consumption and preparation and NAT2 phenotype: ORs and 95% CIs[a]

Variable	Categories		Imputed NAT2 phenotype	
	Men	Women	Slow acetylator	Intermediate or rapid acetylator
Amount of red meat[b]	≤2.6	≤1.7	1.0	1.2 (0.9–1.6)
	2.7–4.6	1.8–3.2	1.1 (0.8–1.4)	1.2 (0.9–1.6)
	4.7–8.0	3.3–5.4	1.2 (0.9–1.6)	1.4 (1.0–1.8)
	>8.0	>5.4	1.0 (0.8–1.4)	1.1 (0.8–1.5)
Frequency of fried, broiled, baked, or barbecued red meat[c]	≤52	≤52	1.0	1.1 (0.8–1.3)
	53–104	53–104	1.1 (0.8–1.4)	1.2 (0.7–1.5)
	105–156	105–156	1.2 (0.9–1.5)	1.1 (0.7–1.5)
	>156	>156	1.0 (0.8–1.2)	1.2 (0.9–1.6)
Preferred doneness of red meat	Rare/medium rare		1.0	0.9 (0.7–1.2)
	Medium-well done		1.0 (0.8–1.3)	1.1 (0.9–1.5)
	Well done		1.0 (0.8–1.3)	1.2 (1.0–1.5)
Frequency of red meat drippings[c]	0	0	1.0	1.0 (0.8–1.2)
	1–12	1–12	0.8 (0.6–1.0)	0.9 (0.7–1.2)
	13–52	13–36	0.9 (0.7–1.1)	1.0 (0.7–1.3)
	>52	>36	0.8 (0.7–1.1)	1.0 (0.7–1.3)
Red meat Mutagen Index	Low		1.0	1.0 (0.8–1.4)
	2		0.9 (0.7–1.2)	0.9 (0.7–1.3)
	3		1.0 (0.8–1.3)	1.0 (0.8–1.3)
	High		1.0 (0.8–1.3)	1.3 (1.0–1.7)
Amount of processed meat[b]	≤0.6	≤0.3	1.0	1.1 (0.8–1.5)
	0.7–1.4	0.4–0.7	1.2 (0.9–1.6)	1.2 (0.9–1.6)
	1.5–2.6	0.8–1.5	1.3 (1.0–1.7)	1.2 (0.9–1.6)
	>2.6	>1.5	1.1 (0.8–1.5)	1.5 (1.1–2.0)
Amount of poultry[b]	≤0.6	≤0.7	1.0	1.2 (0.9–1.6)
	0.7–1.3	0.8–1.4	0.9 (0.7–1.2)	1.2 (0.9–1.6)
	1.4–2.4	1.5–2.3	1.1 (0.9–1.4)	1.1 (0.8–1.4)
	>2.4	>2.3	1.1 (0.8–1.4)	1.1 (0.8–1.4)
Frequency of fried, broiled, baked, or barbecued white meat[c]	≤52	≤52	1.0	1.2 (1.0–1.5)
	53–72	53–104	1.0 (0.8–1.4)	1.0 (0.7–1.3)
	73–104	105–156	1.0 (0.8–1.3)	1.1 (0.9–1.5)
	>104	>156	1.3 (1.0–1.6)	1.1 (0.9–1.5)
Frequency of use of white meat drippings[c]	0		1.0	1.0 (0.9–1.2)
	1–12		0.9 (0.7–1.1)	1.1 (0.8–1.4)
	>12		1.0 (0.8–1.3)	1.2 (0.9–1.5)
White meat Mutagen Index	Low		1.0	1.1 (0.8–1.4)
	2		1.1 (0.8–1.4)	1.3 (1.0–1.7)
	3		1.1 (0.8–1.4)	1.1 (0.8–1.4)
	High		1.2 (1.0–1.6)	1.2 (0.9–1.6)
Total meat Mutagen Index	Low		1.0	1.0 (0.8–1.4)
	2		1.1 (0.8–1.4)	1.1 (0.9–1.5)
	3		1.1 (0.8–1.4)	1.1 (0.9–1.5)
	High		1.2 (0.9–1.5)	1.3 (1.0–1.7)

[a] Adjusted for age, BMI, calories, dietary fiber, lifetime physical activity, and usual no. of cigarettes smoked.
[b] Servings per week.
[c] Frequency per year.

related to cooking time, internal temperature, and degree of surface browning (58). Nonetheless, no marked associations were observed in this study for the frequency of consumption of fried, broiled, baked, or barbecued meat and use of drippings. This finding may be related to the small range in habits of the participants: most people reported consuming meat medium-rare to medium-well done. The actual browning of the meat surface and exact cooking temperature were not assessed in this study. The use of in-person questionnaire administration ensured that detailed and complete information on diet, physical activity, and other potentially confounding variables was obtained.

The finding for processed meat, although consistent with some other recent observations on colorectal cancer, does not provide data on the role of heterocyclic amines but is consistent with a role for nitrosamines (59).

This study does not provide strong evidence that genetically determined rapid metabolizers of potentially carcinogenic compounds in well-done meat are at increased risk of colon cancer. The *NAT2* genotype frequencies observed in our study are similar to those observed by Cascorbi *et al.* (48) in a Caucasian German population (844 unrelated subjects) and in our own parallel study on colorectal adenomatous polyps (60). It should be noted that studies that suggest an association between NAT2 status and colorectal cancer risk were mainly small studies in which phenotyping methods were used to assess NAT2 acetylation status (33, 34). Most (36, 37) but not all (61) recent studies using genotyping

Table 6 Meat consumption and preparation and *GSTM1* genotype: ORs and 95% CIs[a]

Variable	Men	Women	*GSTM1* genotype	
			Present	Null
Amount of red meat[b]	≤2.6	≤1.7	1.0	1.1 (0.8–1.4)
	2.7–4.6	1.8–3.2	1.2 (0.9–1.5)	1.0 (0.7–1.4)
	4.7–8.0	3.3–5.4	1.3 (0.9–1.7)	1.2 (0.9–1.6)
	>8.0	>5.4	1.1 (0.8–1.5)	1.0 (0.7–1.3)
Frequency of fried, broiled, baked, or barbecued red meat[c]	≤52	≤52	1.0	1.0 (0.8–1.2)
	53–104	53–104	1.1 (0.9–1.5)	1.0 (0.8–1.3)
	105–156	105–156	1.4 (1.0–1.8)	1.0 (0.8–1.3)
	>156	>156	0.9 (0.7–1.2)	1.1 (0.8–1.4)
Preferred doneness of red meat	Rare/medium rare		1.0	0.9 (0.7–1.2)
	Medium-well done		1.1 (0.8–1.4)	1.1 (0.8–1.4)
	Well done		1.2 (0.9–1.6)	1.1 (0.8–1.4)
Frequency of red meat drippings[c]	0	0	1.0	1.1 (0.9–1.4)
	1–12	1–12	0.9 (0.7–1.2)	0.8 (0.6–1.0)
	13–52	13–36	1.1 (0.8–1.4)	0.8 (0.6–1.1)
	>52	>36	1.0 (0.8–1.4)	0.9 (0.7–1.2)
Red meat Mutagen Index	Low		1.0	1.2 (0.8–1.5)
	2		1.0 (0.7–1.4)	0.9 (0.7–1.2)
	3		1.1 (0.9–1.5)	1.0 (0.7–1.3)
	High		1.2 (0.9–1.6)	1.1 (0.9–1.5)
Amount of processed meat[b]	≤0.6	≤0.3	1.0	1.0 (0.8–1.4)
	0.7–1.4	0.4–0.7	1.3 (1.0–1.7)	1.1 (0.8–1.4)
	1.5–2.6	0.8–1.5	1.2 (0.9–1.6)	1.2 (0.9–1.6)
	>2.6	>1.5	1.2 (0.9–1.7)	1.2 (0.9–1.6)
Amount of poultry[b]	≤0.6	≤0.7	1.0	1.0 (0.7–1.3)
	0.7–1.3	0.8–1.4	0.9 (0.6–1.1)	1.1 (0.8–1.4)
	1.4–2.4	1.5–2.3	1.1 (0.9–1.5)	0.9 (0.7–1.2)
	>2.4	>2.3	1.1 (0.8–1.4)	1.0 (0.7–1.3)
Frequency of fried, broiled, baked, or barbecued white meat[c]	≤52	≤52	1.0	1.1 (0.9–1.4)
	53–72	53–104	1.1 (0.7–1.5)	0.9 (0.7–1.3)
	73–104	105–156	1.1 (0.9–1.4)	1.0 (0.8–1.3)
	>104	>156	1.4 (1.1–1.8)	1.1 (0.8–1.4)
Frequency of use of white meat drippings[c]	0		1.0	1.0 (0.8–1.2)
	1–12		1.0 (0.8–1.3)	0.9 (0.7–1.1)
	>12		1.2 (0.9–1.6)	1.1 (0.8–1.4)
White meat Mutagen Index	Low		1.0	1.0 (0.8–1.4)
	2		1.1 (0.9–1.5)	1.3 (1.0–1.7)
	3		1.2 (0.9–1.6)	1.0 (0.8–1.3)
	High		1.4 (1.1–1.9)	1.2 (0.9–1.6)
Total meat Mutagen Index	Low		1.0	1.2 (0.9–1.5)
	2		1.1 (0.9–1.6)	1.1 (0.9–1.5)
	3		1.4 (1.0–1.8)	1.0 (0.8–1.4)
	High		1.4 (1.0–1.8)	1.3 (1.0–1.8)

[a] Adjusted for age, BMI, calories, dietary fiber, lifetime physical activity, and usual no. of cigarettes smoked.
[b] Servings per week.
[c] Frequency per year.

methods confirm our potential null findings for NAT2 status. Indeed, our failure to find an association with the rapid *NAT2* genotype may suggest that there is a greater discrepancy between genotype and phenotype than has been reported previously; nonetheless, current data suggest a very tight correlation (62). Two quite small case-control studies show, however, that the combination of a rapid NAT2 phenotype and frequent consumption of meat may increase risk of colorectal cancer risk (37, 63). In an Australian study (110 cases and 110 controls), meat consumption was observed to be associated with an increased colon cancer risk only among NAT2 rapid acetylators (63). A recently conducted study in north-east England showed that 7.4% of the 174 cases carried one or two wild-type *NAT2* alleles and consumed fried meat more than twice a day, whereas this combination was only found in 1.7% of the 174 population-based controls (37). The prospective Physician's Health Study showed, after 13 years of follow-up, an OR of 1.5 (95% CI = 0.6–3.6) among *NAT2* rapid acetylators consuming meat more than once a day as compared to rapid acetylators eating meat 0.5 or fewer times a day (36). However, stronger associations were observed among men older than 60 years old (OR = 4.1, 95% CI = 1.0–17.5, including nine cases and five controls; Ref. 36). Our study does not confirm this age difference. That study also noted a higher risk for colorectal cancer in those who had a high meat intake and who were both rapid NAT1 and rapid NAT2 acetylators. Currently, we do not have data on NAT1 status in our population.

A homozygous deletion of the *GSTM1* gene was present in 53% of the control population in our study, similar to frequencies observed in other Caucasian populations (64). Our study does not confirm the findings of small studies in China and

Japan, suggesting an increased risk of colon cancer for those with the homozygous GSTM1 deletion (65, 66). Lin *et al.* (67) showed no greater risk for colorectal adenomas in those who were *GSTM1* null, consistent with the findings reported here for cancer. Most recently, these investigators have shown that isothiocyanate-rich broccoli is inversely associated with risk of adenomas only in those with the null genotype, which is plausibly the result of a compensatory induction of other phase II enzymes (68).

Exposure to heterocyclic amines might also be especially high among rapid oxidizers, due to genetic differences of the *CYP1A2* gene. However, CYP1A2 is observed to be inducible by a diet high in heterocyclic amines and exhibits a relatively modest intraindividual correlation (69). The genotypic polymorphisms have not been reported thus far. Other enzymes, such as those encoded by *N*-acetyltransferase 1 (*NAT1*) and GST T1 (*GSTT1*) may also influence colon cancer risk. We and others are currently exploring these possibilities.

In summary, this large case-control study provides little support for the hypothesis that there is an increased risk of colon cancer among those with relatively high meat consumption. Genetic differences in the activity of enzymes (NAT2 and GSTM1) metabolizing some of the relevant carcinogenic compounds were neither associated with risk directly nor did they modify the risks associated with meat variables.

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Appendix

NAT2 imputed phenotypes and corresponding genotypes among colon cancer cases and population-based controls

NAT 2 imputed phenotypes and genotypes	Men, *n* (%)		Women, *n* (%)	
	Cases	Controls	Cases	Controls
Mutant/mutant: slow	536	603	394	553
*NAT2*5A/*5A*	1 (0.2)	0	1 (0.2)	1 (0.2)
*NAT2*5A/*5B,C*	14 (2.6)	20 (3.3)	21 (5.3)	21 (3.8)
*NAT2*5A/*6A,B*	15 (2.8)	13 (2.2)	10 (2.5)	11 (2.0)
*NAT2*5A/*7A,B*	2 (0.4)	1 (0.2)	0	2 (0.4)
*NAT2*5B,C/*5B,C*	168 (31.3)	192 (31.8)	118 (30.0)	157 (28.4)
*NAT2*5B,C/*6A,B*	119 (42.7)	242 (40.1)	168 {42.6)	212 (38.3)
*NAT2*5B,C/*7A,B*	20 (3.7)	33 (5.5)	9 (2.3)	28 (5.1)
*NAT2*6A,B/*6A,B*	63 (11.7)	85 (14.1)	51 (12.9)	103 (18.6)
*NAT2*6A,B/*7A,B*	17 (3.2)	12 (0.2)	12 (3.1)	11 (2.0)
*NAT2*7A,B/*7A,B*	1 (0.2)	3 (0.5)	1 (0.2)	0
*NAT2*14/*14*	1 (0.2)	0	1 (0.2)	0
*NAT2*14/*5A*	0	0	0	0
*NAT2*14/*5B,C*	2 (0.4)	1 (0.2)	0	4 (0.7)
*NAT2*14/*6A,B*	3 (0.6)	1 (0.2)	2 (0.5)	3 (0.5)
*NAT2*14/*7A,B*	0	0	0	0
Wild type/mutant: intermediate	323	361	281	322
*NAT2*4/*5A*	6 (1.9)	14 (3.9)	12 (4.3)	12 (3.7)
*NAT2*4/*5B,C*	166 (51.4)	200 (55.4)	152 (54.1)	180 (55.4)
*NAT2*4/*6A,B*	129 (39.9)	124 (34.3)	88 (31.3)	111 (34.5)
*NAT2*4/*7A,B*	7 (2.2)	9 (2.5)	10 (3.6)	9 (2.8)
*NAT2*4/*14*	2 (0.6)	0	1 (0.4)	0
*NAT2*12A,B/*5A*	0	0	0	0
*NAT2*12A,B/*5B,C*	4 (1.2)	7 (1.9)	10 (3.6)	4 (1.2)
*NAT2*12A,B/*6A,B*	7 (2.2)	6 (1.9)	6 (2.1)	5 (1.6)
*NAT2*12A,B/*7A,B*	1 (0.3)	1 (0.3)	2 (0.7)	1 (0.3)
*NAT2*12A,B/*14*	1 (0.3)	0	0	0
Wild type/wild type: rapid	53	72	37	52
*NAT2*4/*4*	49 (92.5)	64 (88.9)	31 (83.8)	41 (86.5)
*NAT2*12A,B/*12A,B*	0	1 (1.4)	0	0
*NAT2*4/*12A,B*	4 (7.5)	7 (9.7)	6 (16.2)	7 (13.5)

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