Using gene-environment interaction analyses to clarify the role of well-done meat and heterocyclic amine exposure in the etiology of colorectal polyps[1–3]

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ABSTRACT

Background: The role of well-done meat intake and meat-derived mutagen heterocyclic amine (HCA) exposure in the risk of colorectal neoplasm has been suggested but not yet established.

Objective: With the use of gene-environment interaction analyses, we sought to clarify the association of HCA exposure with colorectal polyp risk.

Design: In a case-control study including 2057 colorectal polyp patients and 3329 controls, we evaluated 16 functional genetic variants to construct an HCA-metabolizing score. To derive dietary HCA-exposure amount, data were collected regarding dietary intake of meat by cooking method and degree of doneness.

Results: A 2-fold elevated risk associated with high red meat intake was found for colorectal polyps or adenomas in subjects with a high HCA-metabolizing risk score, whereas the risk was 1.3- to 1.4-fold among those with a low risk score (*P*-interaction $\leq$ 0.05). The interaction was stronger for the risk of advanced or multiple adenomas, in which an OR of 2.8 (95% CI: 1.8, 4.6) was observed for those with both a high HCA-risk score and high red meat intake (*P*-interaction = 0.01). No statistically significant interaction was found in analyses that used specific HCA exposure derived from dietary data.

Conclusion: High red meat intake is associated with an elevated risk of colorectal polyps, and this association may be synergistically modified by genetic factors involved in HCA metabolism. *Am J Clin Nutr* 2012;96:1119–28.

INTRODUCTION

High meat intake has been shown to be associated with an increased risk of colorectal cancer (1, 2). Heterocyclic amines (HCAs)[4] are mutagens found in meats cooked at high temperatures (3–5), which may explain part of the positive association between meat intake and colorectal cancer risk. Colorectal adenomatous polyps (adenomas) are well-established precursors of colorectal cancer (6, 7). Recent studies also suggest that some hyperplastic polyps may progress to cancer (8). Several previous observational studies have provided support for a possible association between the risk of colorectal polyps and a high intake of red meat (9–17). It is unclear, however, whether the meat/polyp association is mediated through high exposure to meat-derived mutagens or other constituents in meat. Observational studies, however, have multiple inherent limitations, including potential selection bias and confounding effects. Additional research, such as that using Mendelian randomization analyses, is needed to reduce bias associated with observational studies.

Meat-derived HCAs are procarcinogens, which must be activated by metabolizing enzymes to exert their carcinogenic effects (18–20). Some phase I enzymes [including cytochrome P450 (CYP) 1A2 and CYP1B1] and phase II enzymes [such as sulfotransferases (SULTs) and *N*-acetyltransferases (NATs)] are involved in the bioactivation of HCAs. On the other hand, some phase II enzymes, including UDP-glucuronosyltransferases (UGTs) and glutathione *S*-transferases (GSTs), are responsible for the detoxification of HCAs (20). The aryl hydrocarbon receptor (AhR) is an important xenobiotic signaling mediator that enhances the expression of both phase I and II enzymes (21), which affect HCA metabolism. Therefore, the internal dose of HCA exposure, and the resulting biological effects, is influenced by the balance of enzymes that activate and detoxify HCAs. The amount and function for key HCA-metabolizing enzymes are

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[2] Supported by grants P50CA950103 and R01CA97386 from the National Cancer Institute (NCI). The survey was conducted in part by the Biospecimen and Survey Research Shared Resource, which is supported in part by NCI grant P30CA68485.

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[4] Abbreviations used: ABI, Applied Biosystems; AhR, aryl hydrocarbon receptor; CYP, cytochrome P450; DiMeIQx, 2-amino-3,4,8-trimethylimidazo-[4,5-f]-quinoxaline; GST, glutathione *S*-transferase; HCA, heterocyclic amine; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-f]-quinoxaline; NAT, *N*-acetyltransferase; NSAID, nonsteroidal antiinflammatory drug; PCR, polymerase chain reaction; PhIP, 2-amino-I-methyl-6-phenylimidazo-[4,5-b]-pyridine; SNP, single nucleotide polymorphism; SULT, sulfotransferase; UGT, UDP-glucuronosyltransferase; VA, Veterans Affairs.

Received April 3, 2012. Accepted for publication August 3, 2012.

First published online September 26, 2012; doi: 10.3945/ajcn.112.040345.

determined, in part, by genetic polymorphisms of genes encoding these enzymes. Therefore, the categorization of HCA-metabolizing enzyme genotypes provides a tool to further classify participants into groups with different internal HCA-exposure levels among those with the same external (dietary) HCA-exposure level. In other words, if HCA exposure is causally associated with colorectal polyp risk, we would expect that this association may be modified by genetic polymorphisms of enzymes involved in HCA metabolism, and subjects with a high internal exposure to biologically active HCA, as predicted by both external exposure assessment and HCA-metabolizing pattern, may be at a particularly elevated risk of polyps. We used data and DNA samples collected in the Tennessee Colorectal Polyp Study to test this hypothesis. Because HCA-metabolizing genotypes are established through the random assortment process during gamete formation, they should be independent of external HCA exposure and are unlikely to be related to confounding factors. Therefore, our study is consistent with the Mendelian randomization analysis in studying gene-environment interactions in the risk of diseases, which helps to reduce or even eliminate potential bias associated with observation studies (22–24).

SUBJECTS AND METHODS

Recruitment of study participants

The Tennessee Colorectal Polyp Study is an ongoing colonoscopy-based case-control study conducted in Nashville, Tennessee. Detailed methods used in this study were described elsewhere (15, 25). Eligible participants aged 40–75 y were identified from patients scheduled for colonoscopy at an academic medical center (Vanderbilt University Medical Center) and a Veterans Affairs (VA) medical center (Tennessee Valley Health System, Nashville, TN) between 1 February 2003 and 26 March 2010. The study was approved by the Institutional Review Boards of all participating institutions.

Excluded from our study were participants who had genetic colorectal cancer syndromes or a history of inflammatory bowel disease, adenomatous polyps, or any cancer other than nonmelanoma skin cancer. Of 10,074 eligible participants, 7330 (72.8%) provided written informed consent, of whom 6331 (86.4% of responders) completed a telephone interview. On the basis of the colonoscopy and pathologic findings, polyp cases were categorized as follows: cases with hyperplastic polyps only and cases with any adenomas (including patients with adenoma only or synchronous adenoma and hyperplastic polyp). Eligible controls were participants who had received a complete colonoscopy reaching the cecum and were found to be polyp-free. Twenty-four cases were excluded because of missing data. Of those with completed interviews, 5386 participants were genotyped. The current analyses included 1527 cases with any adenomas, 530 cases with hyperplastic polyps only, and 3329 polyp-free controls. On the basis of the endoscopic report, advanced adenomas were defined as adenomas with a diameter $\geq$1 cm, high-grade dysplasia, or tubulovillous or villous morphology.

Assessment of dietary and other lifestyle factors

In the telephone interview, participants were asked about medication use, demographics, medical history, and selected lifestyle factors; the interview included the use of a meat-specific questionnaire regarding 11 food items as described in detail previously (15, 26). This meat-specific questionnaire was developed to assess carcinogen exposure from intake of cooked meat (27). The questionnaire has been used in many previous studies (11, 14, 16, 28, 29), including our previous study of breast cancer (30). Data regarding meat-intake frequency, usual portion size, and degree of doneness were obtained for 11 meat items by cooking method [oven-broiled or oven-baked, grilled or barbecued, pan fried, deep fried (for chicken and fish), and all other ways]. Participants were asked to report their usual preference of meat doneness by using a series of color photographs. Information about cigarette smoking, alcohol drinking, and nonsteroidal antiinflammatory drug (NSAID) use was also collected in the questionnaires. Regular cigarette smoking was defined as smoking $\geq$1 cigarette/d for $\geq$3 mo continuously. Regular alcohol drinking was defined as consumption of $\geq$5 drinks/wk for 12 mo continuously. Regular NSAIDs users were defined as those who used NSAIDs $\geq$3 times/wk for $\geq$12 mo continuously. All cutoffs for dietary intake were based on quartile distributions in control participants.

Genetic variant selection and genotyping assays

For this study, we selected 10 key enzymes involved in HCA metabolism. These enzymes are categorized into HCA activation and detoxification (**Table 1**). Through an extensive literature search, we identified 24 known functional genetic variants in genes involved in the HCA-metabolism pathway (Table 1). Of these, 22 were successfully genotyped. Variants that failed in the genotyping were C1095A (3′UTR, rs15561) and T1088A (3′UTR, rs1057126) in the *NAT1* gene. Four *NAT1* single nucleotide polymorphisms (SNPs), ie, C97T (R33Stop), C190T (R64W), C620T (T207I), and A752T (D251V) were monomorphic in our population and thus were excluded. As a result, 2 NAT1 SNPs [ie, *NAT1*14* (G560A, rs4986782) and *NAT1*15* (C559T, rs5030839)] were left, which could not be used to impute NAT1 phenotype; thus, they were excluded from the analysis. The 16 genetic variants included in the analysis were *AhR* (G1661A, rs2066853), *CYP1A2*1K* (C163A, rs762551), *CYP1B1*3* (G4329C, rs1056836), *CYP1B1*4* (A4393G, rs1800440), *SULT1A1*2* (G638A, rs9282861), *EPHX1* (T337C, rs1051740), *UGT1A7*9* (G343A, rs61261057), *GSTM1* homozygous deletion, *GSTT1* homozygous deletion, and 7 *NAT2* SNPs [(C282T, rs1041983), (A803G, rs1208), (C481T, rs1799929), (G590A, rs1799930), (G857A, rs1799931), (G191A, rs1801279), and (T341C, rs1801280)].

Genotyping assays were conducted by using genomic DNA extracted from blood or buccal cells (42). All allelic gene polymorphisms were assessed by TaqMan OpenArray system. The TaqMan OpenArray Assay-on-Demand reagents were available from Applied Biosystems (ABI) for all SNPs except *NAT2* G191A (*rs1801279*). The primers for *NAT2* G191A (*rs1801279*) polymorphisms are self-designed and synthesized by ABI (primers were GGAGTTGGGCTTAGAGGCTATTTT and CAGAAGTTGATTGACCTGGAGACA; probes were VIC-CCACCCCGGTTTC and FAM-CCCACCCTGGTTTC). The primers and probes for these SNPs were preloaded by ABI. DNA sample (2.5 μL) and TaqMan Universal PCR Master Mix (2.5 μL) were mixed in a 384-well plate. Polymerase chain reaction (PCR) was performed, consisting of an initial denaturation

TABLE 1
Polymorphisms in selected heterocyclic amine metabolizing genes and their effect on enzyme function

Gene	Variant (alleles)[1,2]	Risk allele frequency[3]	Amino acid change	Effect of risk allele on metabolizing enzymatic activity
Activation enzymes				
AHR	rs2066853(G/**A**)	0.183	R554K	Higher inducibility (21)
CYP1A2	rs762551 (C/**A**)	0.716	NA	Increasing enzymatic activity (31)
CYP1B1	rs1056836 (**G**/C)	0.532	V432L	Increasing mRNA expression (32)
	rs1800440 (**A**/G)	0.827	N453S	Increasing enzymatic activity (33)
NAT2[4]	rs1041983 (C/**T**)	0.335	Y94Y	The combined effect of all 7 SNPs was categorized as slow (reference), intermediate, and rapid acetylation phenotypes (increasing enzymatic activity) (34, 35)
	rs1208 (A/G)	0.426	R268K	
	rs1799929 (C/**T**)	0.409	L161L	
	rs1799930 (G/**A**)	0.711	R197Q	
	rs1799931 (G/**A**)	0.048	G286E	
	rs1801279 (G/**A**)	0.008	R64Q	
	rs1801280 (C/T)	0.570	I114T	
SULT1A1	rs9282861 (**G**/A)	0.264	R213H	Increasing enzymatic activity (36, 37)
Detoxification enzymes				
SULT1A1	rs1051740 (T/**C**)	0.290	Y113H	Decreasing enzymatic activity (38)
UGT1A7	rs61261057 (G/**A**)	0.003	G115S	Decreasing enzymatic activity (39)
GSTM1	Homozygous deletion	0.486	Null	Depleted enzymatic activity (40, 41)
GSTT1	Homozygous deletion	0.186	Null	Depleted enzymatic activity (41)

[1] Database of single nucleotide polymorphisms (http://www.ncbi.nlm.nih.gov/SNP/).
[2] Risk allele is highlighted in bold.
[3] Frequency of the risk allele among controls in the Tennessee Colorectal Polyp Study.
[4] Database of single nucleotide polymorphisms (http://louisville.edu/medschool/pharmacology/nat/). *See* Supplemental Table 3 under "Supplemental data" in the online issue for details on phenotype imputation.

step at 93°C for 10 min and 50 cycles at 95°C for 45 s, 94°C for 13 s, and 53°C for 134 s and a post-PCR hold at 25°C for 2 min. The fluorescence imaging of 3 genotyping plates could be performed together with the ABI OpenArray NT Imager. Allele frequencies were determined by ABI OpenArray software (AutoCaller). Laboratory staff members were blinded to the case-control status of the samples. Quality control protocols for genotyping assays were used as described previously (42). Briefly, each 384-well plate contained 4 water blanks, 8 Centre d'Etude du Polymorphisme Humain 1347-02 DNA, and 16 blinded quality-control samples. The blinded quality-control samples were taken from the second tube of study samples included in the study. Quality-control samples were distributed across the 384-well plates. Concordance rate for the blinded quality-control samples was 100% for all of these SNPs. In addition, the DNA of 45 white samples from the HapMap and/or Perlegen projects was purchased from Coriell Cell Repositories (http://locus.umdnj.edu/ccr/) and genotyped for all 20 SNPs. The average consistency rate of the 20 SNPs was 99.3% compared with data from HapMap (http://www.hapmap.org) and Perlegen (http://genome.perlegen.com). Call rates for all SNPs were >95% (mean call rate = 97.0%), and all genotypes were consistent with Hardy-Weinberg equilibrium with $P > 0.05$ in controls.

Statistical analysis

Meat intake and meat-derived mutagen exposure were calculated as previously described (26). Briefly, meats were classified by type (total meat and red meat) for statistical analysis. The software CHARRED (http://www.charred.cancer.gov/), developed by the US National Cancer Institute (27), was used to estimate exposure levels to meat-derived mutagens, including 2-amino-3,8-dimethylimidazo[4,5-f]-quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo-[4,5-f]-quinoxaline (DiMeIQx), 2-amino-I-methyl-6-phenylimidazo-[4,5-b]-pyridine (PhIP), benzo[α]pyrene, and the overall mutageneity index, which measures overall mutageneity by revertant colonies per gram of daily meat intake through a standard Ames test with *Salmonella typhimurium* strain TA98 (43).

Each polymorphism was tested in controls to ensure fitting with Hardy-Weinberg equilibrium. A risk allele was defined as an allele that either increases the activity or amount of an HCA-activation enzyme or reduces the activity or amount of an HCA-detoxification enzyme. Predicted NAT2 phenotype was derived based on a combination of several SNPs provided elsewhere (*see* Supplemental Table S1 under "Supplemental data" in the online issue) and was classified as slow, intermediate, or fast, with a score of 0, 1, or 2, respectively. For 9 additional genetic variants, each participant received a score of 0, 1, or 2 for carrying 0, 1, or 2 risk alleles, respectively (*see* Supplemental Table S2 under "Supplemental data" in the online issue). The HCA-metabolizing score was derived by summing the individual risk score across all enzymes involved in HCA activation (AhR, CYP1A2, CYP1B1, NAT2, and SULT1A1) and detoxification [EPHX1 (epoxide hydrolase), UGT1A7, GSTM1, and GSTT1) with a total possible score of 17. The median overall HCA-metabolizing score is 10 in control subjects, and this score was used to classify subjects into low-risk and high-risk HCA-metabolizing groups. By using a cubic spline regression model, we found that among those in the top quartile group of total meat intake, this score was positively associated with polyp risk (P-trend = 0.090). No association of this score with polyp risk was observed among those in the bottom group, the lower 75% intake of total meat. Because there is no threshold effect for this score, it was reasonable to use the median as the cutoff in our analyses.

General linear models and Mantel-Haenszel chi-square tests were used to compare the distribution of demographic characteristics and known or hypothesized colorectal cancer risk factors between HCA-metabolizing risk groups and between case and control groups, with additional adjustment for age and sex when appropriate. Unconditional logistic regression models were used to estimate ORs and their 95% CIs for the association between genetic and lifestyle risk factors and polyp risk. ORs were adjusted for known or hypothesized colorectal cancer risk factors and selected confounders for colorectal adenoma, which showed significantly different distributions between cases and controls. Variables selected for multivariate analysis included age (40–49, 50–59, 60–64, or ≥65 y), sex, study site (academic medical center or VA medical center), educational attainment (high school or less, some college, college graduate, or graduate or professional education), cigarette smoking status (never, former, current, or pack-years), regular alcohol consumption (never, former, or current), BMI (continuous), regular exercise (yes or no), regular NSAID use (never, former, or current), year of recruitment, recruitment before or after colonoscopy, and total energy intake level. Energy intake level for those who did not provide FFQ information (n = 21) were input with age- (40–49, 50–59, 60–64, or ≥65 y) and sex-specific mean values. P values for the linear trend tests were derived by treating the quartile variable as a continuous variable in the models by assigning 0, 1, 2, and 3 to quartiles 1 through 4, respectively (44). Likelihood ratio tests were used to evaluate multiplicative interactions by comparing the models with and without interaction terms (44). Interaction tests were based on the analysis by using quartile distributions of dietary intake and a dichotomized genetic summary score. P values ≤0.05 (2-sided probability) were considered statistically significant. All analyses were conducted by using SAS statistical software (version 9.2; SAS Institute).

RESULTS

The function of HCA-metabolizing enzymes and functional genetic variants of these enzymes included in this analysis are summarized in Table 1. Highlighted in bold are the risk alleles, which are predicted to increase internal HCA-carcinogen exposure, based on results from in vitro functional studies.

Distributions of selected demographic characteristics and major risk factors for colorectal cancer are presented in **Table 2** for the 2 polyp case groups and the polyp-free controls. The proportion of polyp-free controls was higher in the academic medical center than in the VA medical center. Compared with controls, polyp cases were more likely to be male, smokers, and regular alcohol consumers and less likely to use NSAIDs regularly. Cases also had a higher BMI, lower educational attainment, and higher daily total energy intake than did controls. Cases with any adenoma were older than controls, whereas hyperplastic polyp–only cases were similar in age to controls. Case-control distributions of race and indication for colonoscopy were comparable. Compared with controls, cases consumed a significantly higher amount of total meat and red meat, had a higher exposure to HCAs (MeIQx, PhIP, and DiMeIQx), and had a higher overall meat mutageneity.

No apparent significant association was observed between the summary HCA-metabolizing risk score and the risk of any polyp groups, including patients with all polyps, any adenoma, or hyperplastic polyp only (**Table 3**). Data regarding the association of polyp risk with each of the SNPs in HCA-metabolizing enzymes are presented elsewhere (*see* in Supplemental Table S2 under "Supplemental data" in the online issue). Again, no statistically significant association was found with any of these SNPs.

Estimates of the risk of polyps stratified by HCA-metabolizing risk category and dietary exposure variables are shown in **Table 4**. With the exception of white meat, dietary exposure variables were, in general, more strongly associated with the risk of all polyps combined in those with a high HCA-metabolizing risk (overall score >10) than in those with a low score, and tests for interactions were statistically significant for red meat intake (P = 0.037). A similar pattern of association was observed in the analysis of any-adenoma group (middle panel), in which the modifying effect of HCA-metabolizing risk score was statistically significant for total meat and red meat intake exposure (P-interaction ≤ 0.05 for both). More apparent associations with red meat and HCA-related variables were found in the analysis of more clinically significant adenomas (advanced or multiple adenomas, right column in Table 4) than with the previous 2 case groups. The strongest association was observed for those with both a high HCA-metabolizing score and a high red meat intake (OR: 2.8; 95% CI: 1.8, 4.6; P-interaction = 0.01). No statistically significant finding was observed in analyses of interactions between polyp risk and specific HCA exposure or overall mutageneity index derived by using dietary variants. Analyses conducted among patients with hyperplastic polyps did not show any statistically significant interactions, perhaps because of the small number of cases in that group (data not shown).

DISCUSSION

In this study, we showed that increased consumption of red meat synergistically interacted with genetically controlled HCA-metabolizing pattern to increase colorectal polyp risk. These results suggest that the association between red meat intake and risk of colorectal neoplasia may be mediated through exposure to HCA.

In general, the association of polyp risk with individual HCA-exposure variables and overall meat mutageneity derived from dietary meat intake was weaker than that with red meat intake. One possible explanation may be the presence of considerable measurement errors in HCA-exposure assessment, as determined with a food questionnaire and the CHARRED program. Although no statistically significant interaction was found between HCA-metabolizing patterns and any HCA-exposure variables, we did observe a stronger association of these HCA-exposure variables with polyp risk in subjects with a high HCA-metabolizing risk score than in those with a low risk score. It is possible that the overall interaction test may not be statistically significant in an analysis that includes both significant and nonsignificant point estimates. Small sample size could have contributed to the nonsignificant OR in some subgroups. Interestingly, a marginally significant interaction between MeIQx exposure and HCA-metabolizing score was found for advanced/multiple adenomas. MeIQx was shown to be ~3- to 7-fold more potent than PhIP in carcinogenicity (3). MeIQx is also more strongly associated with cancers than is PhIP (45). Most DiMeIQx and MeIQx were derived from red meat (3). For example, >85% of MeIQx in the

TABLE 2
Selected demographic characteristics and major known risk factors for colorectal cancer by study group: the Tennessee Colorectal Polyp Study, 2003–2010[1]

Characteristic	Controls (n = 3329)	Polyp cases: Any adenoma[2] (n = 1527)	P value[3]	Polyp cases: HPP only (n = 530)	P value[3]
Study site (%)			<0.001		<0.001
Vanderbilt University	72.8	57.5		54.0	
Veterans Affairs	26.2	42.5		46.0	
Age (y)	56.8 ± 7.6[4]	58.8 ± 7.2	<0.001	56.7 ± 7.9	0.823
Sex, female (%)	43.7	24.8	<0.001	30.0	<0.001
Indications for colonoscopy (%)[5]			0.158		0.576
Screening	58.6	57.2		56.1	
Other	41.4	42.8		43.9	
Educational attainment (%)[5]					<0.001
High school or less	24.5	31.0	<0.001	33.0	
Some college	28.7	29.8		31.3	
College graduate	20.3	20.0		18.6	
Graduate or professional education	26.5	19.3		17.1	
Race, white (%)	87.4	87.4	0.977	90.4	0.752
Colorectal cancer, family history (%)[5]	8.4	9.9	0.258	8.1	0.371
Regular cigarette smoking (%)[5]	49.1	61.3	<0.001	72.6	<0.001
Regular alcohol consumption (%)[5]	43.1	47.0	<0.001	50.4	<0.001
BMI (kg/m^2)[6]	28.1	28.8	0.009	28.9	<0.001
Regular exercise (%)[5]	57.5	52.2	<0.001	53.6	<0.001
Regular NSAID use (%)[5]	52.9	50.8	0.398	46.8	0.015
Total energy intake (kcal/d)[5,6]	2031	2101	<0.001	2093	<0.001
Total meat intake (g/d)[6]	110.3	127.3	<0.001	131.2	<0.001
Red meat intake (g/d)[6]	57.5	76.3	<0.001	79.9	<0.001
MeIQx (ng/d)[6]	57.2	75.3	<0.001	78.6	<0.001
PhIP (ng/d)[6]	262.9	300.7	<0.001	297.5	0.018
DiMeIQx (ng/d)[6]	4.8	5.9	<0.001	6.2	<0.001
Overall meat mutageneity index	10,577	12,106	<0.001	13,198	0.002

[1] DiMeIQx, 2-amino-3,4,8-trimethylimidazo-[4,5-f]-quinoxaline; HPP, hyperplastic polyp; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-f]-quinoxaline; NSAID, nonsteroidal antiinflammatory drug; PhIP, 2-amino-I-methyl-6-phenylimidazo-[4,5-b]-pyridine.

[2] Defined as adenoma only or synchronous adenoma and hyperplastic polyp.

[3] Derived from t test for continuous variables and chi-square test for categorical variables. P values reflect the comparison of controls with each of the case groups, and an adjusted P value of 0.025 (0.05/2) was considered statistically significant.

[4] Mean ± SD (all such values).

[5] Standardized by age (40–49, 50–59, 60–64, and ≥65 y) and sex distribution of all study participants.

[6] All values are means.

current study originated from red meat (data not shown). The exposure level to DiMeIQx is very low (Table 2) and perhaps not informative in the analysis of its association with polyp risk. The mutageneity index is a measure of overall revertant colonies from HCA, benzo[a]pyrene, and other mutagens from well-done meat (27); thus, this index is not a measure of HCA-specific exposure.

TABLE 3
Association between HCA-metabolizing scores and colorectal polyp risk: the Tennessee Colorectal Polyp Study, 2003–2010[1]

HCA-metabolizing score by quartile (score)[2]	Controls (n = 3329)	Polyp cases: All polyps (n = 2057) n	OR (95% CI)[4]	Any adenoma[3] (n = 1527) n	OR (95% CI)[4]	HPP only (n = 530) n	OR (95% CI)[4]
Q1 (5, 9)	1672	1041	1.0 (reference)	776	1.0 (reference)	265	1.0 (reference)
Q2 (10)	751	439	0.9 (0.8, 1.1)	316	0.9 (0.7, 1.0)	123	1.1 (0.8, 1.4)
Q3 (11)	518	325	1.0 (0.9, 1.2)	242	1.0 (0.8, 1.2)	83	1.0 (0.8, 1.4)
Q4 (12, 17)	388	252	1.1 (0.9, 1.3)	193	1.1 (0.9, 1.4)	59	1.0 (0.8, 1.4)
P-trend			0.505		0.465		0.710

[1] HCA, heterocyclic amine; HPP, hyperplastic polyp; Q, quartile.

[2] Defined based on the quartile of overall risk score distribution of controls.

[3] Defined as adenoma only or synchronous adenoma and hyperplastic polyp.

[4] Adjusted for age, sex, study site, educational attainment, red meat intake, smoking, alcohol consumption, BMI, physical activity, regular use of nonsteroidal antiinflammatory drugs, total energy intake, year of recruitment, and recruitment before or after colonoscopy.

TABLE 4
Joint association of dietary intake with HCA-metabolizing risk category (low or high) with polyp risk: the Tennessee Colorectal Polyp Study, 2003–2010[1]

	All polyp cases (n = 2057)				Any adenoma cases[3] (n = 1527)				Advanced or multiple adenoma cases (n = 659)			
	Low risk		High risk		Low risk		High risk		Low risk		High risk	
Intake level[2]	n[4]	OR (95% CI)[5]	n[4]	OR (95% CI)[5]	n[4]	OR (95% CI)[5]	n[4]	OR (95% CI)[5]	n[4]	OR (95% CI)[5]	n[4]	OR (95% CI)[5]
Total meat												
Q1 (low)	264/496	1.0 (Ref)	174/359	1.0 (Ref)	205/496	1.0 (Ref)	139/359	1.0 (Ref)	80/496	1.0 (Ref)	61/359	1.0 (Ref)
Q2	244/473	1.0 (0.8, 1.3)	155/352	0.9 (0.7, 1.2)	185/473	1.0 (0.8, 1.3)	100/352	0.8 (0.6, 1.0)	71/473	1.0 (0.7, 1.5)	47/352	0.9 (0.6, 1.4)
Q3	304/489	1.1 (0.8, 1.3)	237/338	1.3 (1.0, 1.7)	219/489	1.0 (0.8, 1.3)	172/338	1.2 (0.9, 1.7)	84/489	0.9 (0.7, 1.3)	80/338	1.4 (0.9, 2.1)
Q4	390/491	1.1 (0.9, 1.4)	289/331	1.4 (1.1, 1.8)	284/491	1.1 (0.8, 1.4)	223/331	1.3 (1.1, 1.8)	123/491	1.2 (0.8, 1.6)	113/331	1.6 (1.1, 2.3)
P-trend		0.217		0.002		0.555		0.005		0.391		0.004
P-interaction		0.061				0.034				0.044		
Red meat												
Q1 (low)	219/495	1.0 (Ref)	118/370	1.0 (Ref)	165/495	1.0 (Ref)	90/370	1.0 (Ref)	64/495	1.0 (Ref)	35/370	1.0 (Ref)
Q2	241/463	1.1 (0.9, 1.4)	200/358	1.5 (1.2, 2.1)	182/463	1.1 (0.9, 1.5)	145/358	1.5 (1.1, 2.1)	68/463	1.1 (0.7, 1.6)	62/358	1.7 (1.1, 2.8)
Q3	297/489	1.2 (0.9, 1.5)	216/333	1.6 (1.2, 2.1)	219/489	1.1 (0.9, 1.5)	156/333	1.5 (1.1, 2.1)	82/489	1.1 (0.7, 1.5)	76/333	2.0 (1.3, 3.3)
Q4	445/502	1.4 (1.1, 1.7)	321/319	2.0 (1.5, 2.7)	327/502	1.3 (1.0, 1.7)	243/319	2.0 (1.5, 2.8)	144/502	1.4 (1.0, 2.0)	128/319	2.8 (1.8, 4.6)
P-trend		0.012		<0.001		0.037		<0.001		0.055		0.001
P-interaction		0.037				0.035				0.010		
White meat												
Q1 (low)	326/517	1.0 (Ref)	263/366	1.0 (Ref)	250/517	1.0 (Ref)	200/366	1.0 (Ref)	103/517	1.0 (Ref)	104/366	1.0 (Ref)
Q2	354/470	1.2 (1.0, 1.5)	194/358	0.8 (0.7, 1.1)	261/470	1.2 (1.0, 1.6)	137/358	0.8 (0.6, 1.1)	114/470	1.4 (1.0, 1.9)	59/358	0.7 (0.5, 1.0)
Q3	266/476	1.0 (0.8, 1.3)	175/327	0.9 (0.7, 1.2)	196/476	1.0 (0.8, 1.3)	129/327	0.9 (0.7, 1.3)	72/476	0.9 (0.7, 1.3)	61/327	0.9 (0.6, 1.3)
Q4	256/486	0.9 (0.7, 1.2)	223/329	1.1 (0.9, 1.5)	186/486	0.9 (0.7, 1.1)	168/329	1.2 (0.9, 1.5)	69/486	0.8 (0.6, 1.2)	77/329	1.1 (0.7, 1.6)
P-trend		0.238		0.246		0.178		0.257		0.114		0.528
P-interaction		0.113				0.093				0.133		
MeIQx												
Q1 (low)	250/524	1.0 (Ref)	146/397	1.0 (Ref)	187/524	1.0 (Ref)	107/397	1.0 (Ref)	73/524	1.0 (Ref)	46/397	1.0 (Ref)
Q2	239/469	1.0 (0.8, 1.3)	186/335	1.4 (1.0, 1.8)	183/469	1.1 (0.8, 1.4)	136/335	1.4 (1.0, 1.9)	72/469	1.0 (0.7, 1.5)	67/335	1.5 (1.0, 2.4)
Q3	314/482	1.2 (1.0, 1.5)	229/319	1.5 (1.2, 2.0)	228/482	1.2 (0.9, 1.6)	169/319	1.6 (1.2, 2.1)	89/482	1.2 (0.8, 1.6)	71/319	1.6 (1.0, 2.5)
Q4	399/474	1.2 (1.0, 1.5)	294/329	1.6 (1.2, 2.1)	295/474	1.2 (0.9, 1.5)	222/329	1.6 (1.2, 2.2)	124/474	1.2 (0.8, 1.6)	117/329	1.9 (1.3, 2.9)
P-trend		0.033		0.001		0.088		0.002		0.277		0.003
P-interaction		0.164				0.105				0.060		
PhIP												
Q1 (low)	279/526	1.0 (Ref)	184/395	1.0 (Ref)	206/526	1.0 (Ref)	142/395	1.0 (Ref)	81/526	1.0 (Ref)	72/395	1.0 (Ref)
Q2	245/474	1.0 (0.8, 1.2)	205/329	1.3 (1.0, 1.6)	184/474	1.0 (0.8, 1.3)	148/329	1.2 (0.9, 1.7)	73/474	1.0 (0.7, 1.5)	68/329	1.2 (0.8, 1.8)
Q3	331/457	1.3 (1.1, 1.6)	217/345	1.2 (1.0, 1.6)	249/457	1.4 (1.1, 1.8)	154/345	1.2 (0.9, 1.6)	101/457	1.5 (1.0, 2.1)	70/345	1.2 (0.8, 1.7)
Q4	347/492	1.2 (0.9, 1.5)	249/311	1.5 (1.1, 1.9)	254/492	1.2 (0.9, 1.5)	190/311	1.5 (1.1, 2.0)	103/492	1.2 (0.9, 1.7)	91/311	1.5 (1.0, 2.2)
P-trend		0.028		0.008		0.026		0.013		0.073		0.049
P-interaction		0.397				0.489				0.621		
DiMeIQx												
Q1 (low)	270/530	1.0 (Ref)	193/391	1.0 (Ref)	206/530	1.0 (Ref)	145/391	1.0 (Ref)	77/530	1.0 (Ref)	70/391	1.0 (Ref)
Q2	269/459	1.2 (0.9, 1.5)	192/347	1.0 (0.8, 1.3)	196/459	1.2 (0.9, 1.5)	144/347	1.1 (0.8, 1.4)	84/459	1.3 (0.9, 1.9)	57/347	0.9 (0.6, 1.3)
Q3	284/475	1.1 (0.9, 1.4)	183/324	1.0 (0.8, 1.3)	212/475	1.1 (0.9, 1.5)	132/324	1.0 (0.7, 1.3)	85/475	1.2 (0.8, 1.7)	70/324	1.0 (0.7, 1.6)
Q4	379/485	1.3 (1.0, 1.6)	287/318	1.4 (1.1, 1.8)	279/485	1.3 (1.0, 1.6)	213/318	1.4 (1.1, 1.9)	112/485	1.2 (0.9, 1.7)	104/318	1.4 (0.9, 2.0)
P-trend		0.041		0.011		0.080		0.028		0.307		0.047
P-interaction		0.418				0.438				0.284		

(Continued)

TABLE 4 (*Continued*)

	All polyp cases (n = 2057)				Any adenoma cases[3] (n = 1527)				Advanced or multiple adenoma cases (n = 659)			
	Low risk		High risk		Low risk		High risk		Low risk		High risk	
Intake level[2]	n[4]	OR (95% CI)[5]	n[4]	OR (95% CI)[5]	n[4]	OR (95% CI)[5]	n[4]	OR (95% CI)[5]	n[4]	OR (95% CI)[5]	n[4]	OR (95% CI)[5]
Overall mutageneity index												
Q1 (low)	258/526	1.0 (Ref)	158/396	1.0 (Ref)	198/526	1.0 (Ref)	121/396	1.0 (Ref)	75/526	1.0 (Ref)	58/396	1.0 (Ref)
Q2	265/464	1.2 (0.9, 1.5)	217/338	1.6 (1.2, 2.1)	189/464	1.1 (0.8, 1.4)	152/338	1.5 (1.1, 2.0)	75/464	1.1 (0.8, 1.6)	66/338	1.6 (1.1, 2.5)
Q3	314/481	1.3 (1.0, 1.6)	217/322	1.6 (1.2, 2.1)	238/481	1.3 (1.0, 1.6)	162/322	1.7 (1.2, 2.3)	96/481	1.3 (0.9, 1.9)	85/322	2.1 (1.4, 3.2)
Q4	365/478	1.3 (1.0, 1.6)	263/324	1.6 (1.2, 2.1)	268/478	1.2 (1.0, 1.6)	199/324	1.6 (1.2, 2.2)	112/478	1.3 (0.9, 1.9)	92/324	1.7 (1.1, 2.5)
P-trend		0.015		0.001		0.027		0.002		0.064		0.009
P-interaction		0.268				0.232				0.334		

[1] The median overall HCA-metabolizing score is 10 in control subjects. An overall HCA-metabolizing score ≤10 indicates low risk, and an overall HCA-metabolizing score >10 indicates high risk. DiMeIQx, 2-amino-3,4,8-trimethylimidazo-[4,5-f]-quinoxaline; HCA, heterocyclic amine; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-f]-quinoxaline; PhIP, 2-amino-1-methyl-6-phenylimidazo-[4,5-b]-pyridine; Q, quartile; Ref, reference.

[2] Cutoff for dietary intake was the quartile intake level of control subjects.

[3] Defined as adenoma only or synchronous adenoma and hyperplastic polyp.

[4] Reflects the number of cases/controls; counts may not sum to the total because of missing data.

[5] Adjusted for age, sex, study site, educational attainment, smoking, alcohol consumption, BMI, physical activity, regular use of nonsteroidal antiinflammatory drugs, total energy intake, year of recruitment, and recruitment before or after colonoscopy.

Previous studies evaluated only a few HCA-metabolizing genetic variants and yielded inconsistent results for a potential modifying effect of genetic factors on the association of HCA exposure with adenoma (46–52) and colorectal cancer risks (53–65). The largely null findings reported from previous studies are not surprising because each genetic variant in the HCA-metabolizing pathway plays a small role in the activation or detoxification of HCAs. Therefore, it is important to combine information from multiple genetic variants to capture the HCA-metabolizing pattern of each individual. Additional functional variants likely will be identified in the future in the HCA-metabolizing pathway, which could improve the classification of participants into HCA-metabolizing risk categories. This means the true synergistic effect of genetic factors and HCA exposure could be stronger than what we observed in this study.

As discussed previously, observational studies may suffer from various biases; thus, it is difficult to make causal inference based on results from observational studies alone. It is unlikely that randomized intervention trials would be conducted to directly evaluate the association of HCA exposure and cancer risk. Mendelian randomization analysis provides unique opportunities for a nature's randomization trial, because it uses the random assortment of genetic variants during gamete formation. This is analogous to the random assignment of patients to placebo or treatment arms in a clinical intervention trial; thus, the analysis minimizes and even eliminates potential bias associated with observational studies (22). As discussed recently by Smith (24), gene-environment interaction analyses can be interpreted within Mendelian randomization when certain criteria are met. Our study, in general, fulfilled these criteria:

1) The genotypic profile, as summarized by the HCA-metabolizing risk score, was independent of factors that may confound the association between meat intake levels or HCA-exposure levels and risk of colorectal polyps. We evaluated this criterion by analyzing the distribution of demographic characteristics and risk factors presented in Table 1 by HCA-metabolizing risk categories (*see* Supplemental Table S3 under "Supplemental data" in the online issue). Our results show no differences between the low and high HCA-metabolizing risk categories regarding distributions of almost all demographic characteristics and risk factors. These findings support the successful Mendelian randomization of our study participants.

2) The HCA genetic-risk profile must only be related to risk of disease through the exposure of interest and not through other pathways. Because many metabolizing genes are pleiotropic, they may be involved in the metabolism of other carcinogens. We selected functional SNPs involved in HCA metabolism and combined them to construct the HCA-specific metabolizing pathway. Because it is highly unlikely that all of the HCA-metabolizing SNPs selected are also involved in the metabolism of another substrate, the metabolizing pathway derived in this study should be more HCA specific than any other substrates. Therefore, the HCA-metabolizing risk score constructed in this study should be more specific for HCA metabolism than for any single variant of HCA-metabolizing enzymes.

We also evaluated the interaction of HCA-metabolizing risk score with cigarette smoking, NSAID use, alcohol intake, BMI,

and physical activity. We found no interactions between these factors and polyp risk. Thus, it is unlikely that the potential pleiotropic effects of certain genes could appreciably affect our study results. Finally, on the basis of HCA-metabolizing patterns, the HCA-metabolizing risk score derived from this study should affect the internal effective dose of HCA exposure and thus can further define internal exposure status for those with the same external exposure level of HCAs. Interestingly, the association of cancer risk with aromatic amines or HCA and carcinogen-metabolizing enzymes is among the examples provided by Smith (24) and by Thomas and Conti (23) for Mendelian randomization analyses of gene-environment interactions. However, residual bias might remain in Mendelian randomization analyses. Therefore, additional studies are needed to further clarify the association of red meat intake and HCA exposure in the risk of colorectal tumors.

This study's focus on precursors for colorectal cancer, ie, colorectal polyps, eliminates possible survival bias commonly encountered in cancer case-control studies as a result of failure to recruit patients with a short survival time. Other strengths of this study include the use of colonoscopy to define patient groups, use of a questionnaire specifically designed to capture intake patterns of well-done meat and meat-carcinogen exposures, and large sample size. With questions regarding meat intake by cooking method and degree of doneness, the questionnaire used in this study collects data more relevant for estimating HCA intake than a typical food-frequency questionnaire.

Because all polyps were benign lesions, recall bias and any subsequent lifestyle change after polyp diagnosis is unlikely to be substantial. Most participants (87.8%) were recruited before colonoscopy, and thus before polyp diagnosis, which reduces possible selection bias. Exclusion of participants recruited after colonoscopy (n = 894) did not appreciably change the associations observed. Response bias is always a possible limitation when response rates are not optimal; however, the diagnosis of polyps cannot influence the genotype of study participants, and the genotype is unlikely to be related to HCA external exposure, ie, dietary meat intake. As discussed previously, these potential biases were reduced in our study through Mendelian randomization analysis.

Our findings suggest that genetic variants in metabolic enzymes may modify the association of dietary meat intake and meat-derived mutagen exposures with colorectal polyp risk. Many explanations have been proposed for the association between meat intake and polyp risk (such as meat-derived fat, iron, and HCA). However, by defining the HCA-specific metabolizing risk pattern for each subject, our study specifically implicates a causal role of HCAs in the formation of colorectal cancer precursors. These findings may aid the understanding of colorectal cancer pathogenesis and the development of prevention strategies.

We thank the study participants and research staff of the Tennessee Colorectal Polyp Study for their support of this research.

The authors' responsibilities were as follows—WZ, MJS, QC, and RMN: designed and directed the study; ZF: analyzed the data; QC and GL: directed the genotyping; GL: conducted the genotyping; ZF, WZ, and MJS: drafted the manuscript; RMN and WES: provided support for the clinical operation; DH: provided support to derive *NAT1* and *NAT2* phenotypes; and ZC and YS: helped with the statistical analysis and data cleaning. All authors reviewed and approved the final draft. None of the authors declared any conflicts of interest.

REFERENCES

1. Larsson SC, Wolk A. Meat consumption and risk of colorectal cancer: a meta-analysis of prospective studies. Int J Cancer 2006;119:2657–64.
2. Zheng W, Lee SA. Well-done meat intake,heterocyclic amine exposure, and cancer risk. Nutr Cancer 2009;61:437–46.
3. Layton DW, Bogen KT, Knize MG, Hatch FT, Johnson VM, Felton JS. Cancer risk of heterocyclic amines in cooked foods: an analysis and implications for research. Carcinogenesis 1995;16:39–52.
4. Sinha R, Knize MG, Salmon CP, Brown ED, Rhodes D, Felton JS, Levander OA, Rothman N. Heterocyclic amine content of pork products cooked by different methods and to varying degrees of doneness. Food Chem Toxicol 1998;36:289–97.
5. Sinha R, Rothman N, Salmon CP, Knize MG, Brown ED, Swanson CA, Rhodes D, Rossi S, Felton JS, Levander OA. Heterocyclic amine content in beef cooked by different methods to varying degrees of doneness and gravy made from meat drippings. Food Chem Toxicol 1998;36:279–87.
6. Stryker SJ, Wolff BG, Culp CE, Libbe SD, Ilstrup DM, MacCarty RL. Natural history of untreated colonic polyps. Gastroenterology 1987;93:1009–13.
7. Anderson WF, Guyton KZ, Hiatt RA, Vernon SW, Levin B, Hawk E. Colorectal cancer screening for persons at average risk. J Natl Cancer Inst 2002;94:1126–33.
8. Leggett B, Whitehall V. Role of the serrated pathway in colorectal cancer pathogenesis. Gastroenterology 2010;138:2088–100.
9. Kono S, Imanishi K, Shinchi K, Yanai F. Relationship of diet to small and large adenomas of the sigmoid colon. Jpn J Cancer Res 1993;84:13–9.
10. Sinha R, Kulldorff M, Chow W-H, Denobile J, Rothman N. Dietary intake of heterocyclic amines, meat-derived mutagenic activity, and risk of colorectal adenomas. Cancer Epidemiol Biomarkers Prev 2001;10:559–62.
11. Gunter MJ, Probst-Hensch NM, Cortessis VK, Kulldorff M, Haile RW, Sinha R. Meat intake, cooking-related mutagens and risk of colorectal adenoma in a sigmoidoscopy-based case-control study. Carcinogenesis 2005;26:637–42.
12. Sinha R, Peters U, Cross AJ, Kulldorff M, Weissfeld JL, Pinsky PF, Rothman N, Hayes RB. Meat, meat cooking methods and preservation, and risk for colorectal adenoma. Cancer Res 2005;65:8034–41.
13. Wu K, Giovannucci E, Byrne C, Platz EA, Fuchs C, Willett WC, Sinha R. Meat mutagens and risk of distal colon adenoma in a cohort of U.S. men. Cancer Epidemiol Biomarkers Prev 2006;15:1120–5.
14. Martínez ME, Jacobs ET, Ashbeck EL, Sinha R, Lance P, Alberts DS, Thompson PA. Meat intake, preparation methods, mutagens and colorectal adenoma recurrence. Carcinogenesis 2007;28:2019–27.
15. Shin A, Shrubsole MJ, Ness RM, Wu H, Sinha R, Smalley WE, Shyr Y, Zheng W. Meat and meat-mutagen intake, doneness preference and the risk of colorectal polyps: the Tennessee Colorectal Polyp Study. Int J Cancer 2007;121:136–42.
16. Ferrucci LM, Sinha R, Graubard BI, Mayne ST, Ma X, Schatzkin A, Schoenfeld PS, Cash BD, Flood A, Cross AJ. Dietary meat intake in relation to colorectal adenoma in asymptomatic women. Am J Gastroenterol 2009;104:1231–40.
17. Rohrmann S, Hermann S, Linseisen J. Heterocyclic aromatic amine intake increases colorectal adenoma risk: findings from a prospective European cohort study. Am J Clin Nutr 2009;89:1418–24.
18. Nebert DW. Role of genetics and drug-metabolism in human cancer risk. Mutat Res 1991;247:267–81.
19. Turesky RJ. Heterocyclic aromatic amine metabolism, DNA adduct formation, mutagenesis, and carcinogenesis. Drug Metab Rev 2002;34:625–50.
20. Turesky RJ. Formation and biochemistry of carcinogenic heterocyclic aromatic amines in cooked meats. Toxicol Lett 2007;168:219–27.
21. Fujii-Kuriyama Y, Mimura J. Molecular mechanisms of AhR functions in the regulation of cytochrome P450 genes. Biochim Biophys Res Commun 2005;338:311–7.
22. Smith GD, Ebrahim S. Mendelian randomization: prospects, potentials, and limitations. Int J Epidemiol 2004;33:30–42.
23. Thomas DC, Conti DV. Commentary: the concept of "Mendelian Randomization." Int J Epidemiol 2004;33:21–5.
24. Smith GD. Mendelian randomization for strengthening causal inference in observational studies. Perspect Psychol Sci 2010;5:527–45.

25. Shrubsole MJ, Wu H, Ness RM, Shyr Y, Smalley WE, Zheng W. Alcohol drinking, cigarette smoking, and risk of colorectal adenomatous and hyperplastic polyps. Am J Epidemiol 2008;167:1050–8.
26. Fu Z, Shrubsole MJ, Smalley WE, Wu H, Chen Z, Shyr Y, Ness RM, Zheng W. Association of meat intake and meat-derived mutagen exposure with the risk of colorectal polyps by histologic type. Cancer Prev Res (Phila) 2011;4:1686–97.
27. Sinha R, Cross A, Curtin J, Zimmerman T, McNutt S, Risch A, Holden J. Development of a food frequency questionnaire module and databases for compounds in cooked and processed meats. Mol Nutr Food Res 2005;49:648–55.
28. Ollberding NJ, Wilkens LR, Henderson BE, Kolonel LN, Le Marchand L. Meat consumption, heterocyclic amines and colorectal cancer risk: the Multiethnic Cohort Study. Int J Cancer 2012;131:E1125–33.
29. Freedman ND, Cross AJ, McGlynn KA, Abnet CC, Park Y, Hollenbeck AR, Schatzkin A, Everhart JE, Sinha R. Association of meat and fat intake with liver disease and hepatocellular carcinoma in the NIH-AARP Cohort. J Natl Cancer Inst 2010;102:1354–65.
30. Zheng W, Gustafson DR, Sinha R, Cerhan JR, Moore D, Hong CP, Anderson KE, Kushi LH, Sellers TA, Folsom AR. Well-done meat intake and the risk of breast cancer. J Natl Cancer Inst 1998;90: 1724–9.
31. Sachse C, Brockmöller J, Bauer S, Roots I. Functional significance of a C→A polymorphism in intron 1 of the cytochrome P450 CYP1A2 gene tested with caffeine. Br J Clin Pharmacol 1999;47:445–9.
32. Landi MT, Bergen AW, Baccarelli A, Patterson DG Jr, Grassman J, Ter-Minassian M, Mocarelli P, Caporaso N, Masten SA, Pesatori AC, et al. CYP1A1 and CYP1B1 genotypes, haplotypes, and TCDD-induced gene expression in subjects from Seveso, Italy. Toxicology 2005;207:191–202.
33. Shimada T, Watanabe J, Kawajiri K, Sutter TR, Guengerich FP, Gillam EM, Inoue K. Catalytic properties of polymorphic human cytochrome P450 1B1 variants. Carcinogenesis 1999;20:1607–13.
34. Hein DW. Molecular genetics and function of NAT1 and NAT2: role in aromatic amine metabolism and carcinogenesis. Mutat Res 2002;506–507:65–77.
35. Metry KJ, Neale JR, Doll MA, Howarth AL, States JC, McGregor WG, Pierce WM Jr, Hein DW. Effect of rapid human N-acetyltransferase 2 haplotype on DNA damage and mutagenesis induced by 2-amino-3-methylimidazo-[4,5-f]quinoline (IQ) and 2-amino-3,8-dimethylimidazo-[4,5-f]quinoxaline (MeIQx). Mutat Res 2011;684:66–73.
36. Raftogianis RB, Wood TC, Otterness DM, VanLoon JA, Weinshilboum RM. Phenol sulfotransferase pharmacogenetics in humans: association of common SULT1A1 alleles with TS PST phenotype. Biochem Biophys Res Commun 1997;239:298–304.
37. Raftogianis RB, Wood TC, Weinshilboum RM. Human phenol sulfotransferases SULT1A2 and SULT1A1—genetic polymorphisms, allozyme properties, and human liver genotype-phenotype correlations. Biochem Pharmacol 1999;58:605–16.
38. Hassett C, Aicher L, Sidhu JS, Omiecinski CJ. Human microsomal epoxide hydrolase: genetic polymorphism and functional expression in vitro of amino acid variants. Hum Mol Genet 1994;3:421–8.
39. Villeneuve L, Girard H, Fortier L-C, Gagne J-F, Guillemette C. Novel functional polymorphisms in the UGT1A7 and UGT1A9 glucuronidating enzymes in Caucasian and African-American subjects and their impact on the metabolism of 7-ethyl-10-hydroxycamptothecin and flavopiridol anticancer drugs. J Pharmacol Exp Ther 2003;307: 117–28.
40. Seidegard J, Vorachek WR, Pero RW, Pearson WR. Hereditary differences in the expression of the human glutathione transferase active on trans-stilbene oxide are due to a gene deletion. Proc Natl Acad Sci USA 1988;85:7293–7.
41. Josephy PD. Genetic variations in human glutathione transferase enzymes: significance for pharmacology and toxicology. Hum Genomics Proteomics 2010;2010:876940.
42. Zheng W, Long J, Gao YT, Li C, Zheng Y, Xiang YB, Wen W, Levy S, Deming SL, Haines JL, et al. Genome-wide association study identifies a new breast cancer susceptibility locus at 6q25.1. Nat Genet 2009;41: 324–8.
43. Ames BN, McCann J, Yamasaki E. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutat Res 1975;31:347–64.
44. Madure M, Greenland S. Tests for trend and dose response: misinterpretations and alternatives. Am J Epidemiol 1992;135:96–104.
45. Sinha R. An epidemiologic approach to studying heterocyclic amines. Mutat Res 2002;506–507:197–204.
46. Ulrich CM, Bigler J, Whitton JA, Bostick R, Fosdick L, Potter JD. Epoxide hydrolase Tyr113His polymorphism is associated with elevated risk of colorectal polyps in the presence of smoking and high meat intake. Cancer Epidemiol Biomarkers Prev 2001;10: 875–82.
47. Ishibe N, Sinha R, Hein DW, Kulldorff M, Strickland P, Fretland AJ, Chow WH, Kadlubar FF, Lang NP, Rothman N. Genetic polymorphisms in heterocyclic amine metabolism and risk of colorectal adenomas. Pharmacogenet Genomics 2002;12:145–50.
48. Chan AT, Tranah GJ, Giovannucci EL, Hunter DJ, Fuchs CS. A prospective study of genetic polymorphisms in the cytochrome P-450 2C9 enzyme and the risk for distal colorectal adenoma. Clin Gastroenterol Hepatol 2004;2:704–12.
49. Tiemersma EW, Voskuil DW, Bunschoten A, Hogendoorn EA, Witteman BJ, Nagengast FM, Glatt H, Kok FJ, Kampman E. Risk of colorectal adenomas in relation to meat consumption, meat preparation, and genetic susceptibility in a Dutch population. Cancer Causes Control 2004;15:225–36.
50. Goode EL, Potter JD, Bamlet WR, Rider DN, Bigler J. Inherited variation in carcinogen-metabolizing enzymes and risk of colorectal polyps. Carcinogenesis 2007;28:328–41.
51. Shin A, Shrubsole MJ, Rice JM, Cai Q, Doll MA, Long J, Smalley WE, Shyr Y, Sinha R, Ness RM, et al. Meat intake, heterocyclic amine exposure, and metabolizing enzyme polymorphisms in relation to colorectal polyp risk. Cancer Epidemiol Biomarkers Prev 2008;17: 320–9.
52. Wang H, Yamamoto JF, Caberto C, Saltzman B, Decker R, Vogt TM, Yokochi L, Chanock S, Wilkens LR, Le Marchand L. Genetic variation in the bioactivation pathway for polycyclic hydrocarbons and heterocyclic amines in relation to risk of colorectal neoplasia. Carcinogenesis 2011;32:203–9.
53. Welfare MR, Cooper J, Bassendine MF, Daly AK. Relationship between acetylator status, smoking, and diet and colorectal cancer risk in the north-east of England. Carcinogenesis 1997;18:1351–4.
54. Chen J, Stampfer MJ, Hough HL, Garcia-Closas M, Willett WC, Hennekens CH, Kelsey KT, Hunter DJ. A prospective study of N-acetyltransferase genotype, red meat intake, and risk of colorectal cancer. Cancer Res 1998;58:3307–11.
55. Le Marchand L, Hankin JH, Wilkens LR, Pierce LM, Franke A, Kolonel LN, Seifried A, Custer LJ, Chang W, Lum-Jones A, et al. Combined effects of well-done red meat, smoking, and rapid N-acetyltransferase 2 and CYP1A2 phenotypes in increasing colorectal cancer risk. Cancer Epidemiol Biomarkers Prev 2001;10:1259–66.
56. Le Marchand L, Hankin JH, Pierce LM, Sinha R, Nerurkar PV, Franke AA, Wilkens LR, Kolonel LN, Donlon T, Seifried A, et al. Well-done red meat, metabolic phenotypes and colorectal cancer in Hawaii. Mutat Res 2002;506–507:205–14.
57. Tiemersma EW, Kampman E. Bueno de Mesquita HB, Bunschoten A, van Schothorst EM, Kok FJ, Kromhout D. Meat consumption, cigarette smoking, and genetic susceptibility in the etiology of colorectal cancer: results from a Dutch prospective study. Cancer Causes Control 2002; 13:383–93.
58. Barrett JH, Smith G, Waxman R, Gooderham N, Lightfoot T, Garner RC, Augustsson K, Wolf CR, Bishop DT, Forman D, et al. Investigation of interaction between N-acetyltransferase 2 and heterocyclic amines as potential risk factors for colorectal cancer. Carcinogenesis 2003;24:275–82.
59. Butler LM, Duguay Y, Millikan RC, Sinha R, Gagné JF, Sandler RS, Guillemette C. Joint effects between UDP-glucuronosyltransferase 1A7 genotype and dietary carcinogen exposure on risk of colon cancer. Cancer Epidemiol Biomarkers Prev 2005;14:1626–32.
60. Chan AT, Tranah GJ, Giovannucci EL, Willett WC, Hunter DJ, Fuchs CS. Prospective study of N-acetyltransferase-2 genotypes, meat intake, smoking and risk of colorectal cancer. Int J Cancer 2005;115: 648–52.
61. Lilla C, Verla-Tebit E, Risch A, Jäger B, Hoffmeister M, Brenner H, Chang-Claude J. Effect of NAT1 and NAT2 genetic polymorphisms on colorectal cancer risk associated with exposure to tobacco smoke and meat consumption. Cancer Epidemiol Biomarkers Prev 2006;15: 99–107.
62. Küry S, Buecher B, Robiou-du-Pont S, Scoul C, Sébille V, Colman H, Le Houérou C, Le Neel T, Bourdon J, Faroux R, et al. Combinations

of cytochrome p450 gene polymorphisms enhancing the risk for sporadic colorectal cancer related to red meat consumption. Cancer Epidemiol Biomarkers Prev 2007;16:1460–7.
63. Butler LM, Millikan RC, Sinha R, Keku TO, Winkel S, Harlan B, Eaton A, Gammon MD, Sandler RS. Modification by N-acetyltransferase 1 genotype on the association between dietary heterocyclic amines and colon cancer in a multiethnic study. Mutat Res 2008;638: 162–74.
64. Cotterchio M, Boucher BA, Manno M, Gallinger S, Okey AB, Harper PA. Red meat intake, doneness, polymorphisms in genes that encode carcinogen-metabolizing enzymes, and colorectal cancer risk. Cancer Epidemiol Biomarkers Prev 2008;17:3098–107.
65. Nöthlings U, Yamamoto JF, Wilkens LR, Murphy SP, Park SY, Henderson BE, Kolonel LN, Le Marchand L. Meat and heterocyclic amine intake, smoking, NAT1 and NAT2 polymorphisms, and colorectal cancer risk in the multiethnic cohort study. Cancer Epidemiol Biomarkers Prev 2009;18:2098–106.
66. Hein DW. N-acetyltransferase 2 genetic polymorphism: effects of carcinogen and haplotype on urinary bladder cancer risk. Oncogene 2006;25:1649–58.