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Xenobiotic Metabolizing Genes, Meat-Related Exposures, and Risk of Advanced Colorectal Adenoma

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The potential for carcinogenic action of meat-related exposures, such as heterocyclic amines (HCAs), polycyclic aromatic hydrocarbons (PAHs), and *N*-nitroso compounds (NOCs) [1, 2], might explain positive associations between red and processed meat intake and colorectal neoplasia [3]. HCAs and PAHs are formed in meats cooked well-done at high temperatures [4] and produce intestinal tumors in rodents [5–7]. NOCs are some of the strongest known chemical carcinogens [2] and induce tumors in both the colon and rectum of numerous animal species [8]. Nitrate and nitrite, which are added to processed meats, can form NOCs [9]. NOCs can also form endogenously in the colon through the conversion of nitrate and nitrite [10], a reaction which is thought to be catalyzed by heme iron from red meat [11, 12].

HCAs, PAHs, and some NOCs are considered procarcinogens, as they require metabolic activation to attain full potential. Phase I and phase II xenobiotic metabolizing enzymes (XMEs) are involved in the activation and detoxification of these substrates [13–17]. Single nucleotide polymorphisms (SNPs) in genes that encode XMEs are hypothesized to alter enzyme expression and function [14], resulting in differential metabolism of xenobiotics between individuals [18]. A number of colorectal adenoma studies have evaluated interactions between XME genes and meat consumption with inconsistent results [19–30], but the majority of these investigated a limited number of genes. In addition, HCAs and PAHs from meat were estimated in just 2 studies [21, 22], while only 1 prior analysis evaluated nitrate/nitrite from processed meat [28].

Utilizing detailed meat-cooking data, we investigated the interaction of HCAs, PAHs, and nitrate/nitrite from meat with several XME gene variants in relation to advanced colorectal adenoma. Examining these interactions with asymptomatic colorectal adenomas, precursors to colorectal cancer [31–33], is valuable as diet should not have been altered by disease. Our analysis expands on findings of increased risk of prevalent colorectal adenoma with well-done red meat and cooking-related mutagens in the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial [34].

Materials and Methods

Study Population

The PLCO Cancer Screening Trial is a randomized, multi-center clinical trial investigating the efficacy of screening for prostate, lung, colorectal and ovarian cancer [35, 36]. Participants aged 55–74 were recruited from 10 centers in the United States. Participants completed a self-administered baseline risk factor questionnaire, a food frequency questionnaire (FFQ), and provided biological samples. The study was approved by the institutional review boards at the National Cancer Institute and the 10 study centers. All participants provided written informed consent.

Cases and controls for this study were selected from participants enrolled in the screening arm of the PLCO Cancer Screening Trial between 1993 and 1999 (n = 77,483). At baseline, participants in the screening arm underwent flexible sigmoidoscopy of the distal colorectum (60 cm). Those with neoplastic lesions were referred for full colonoscopic examination and diagnostic work-up by the participant's personal physician. Trained abstractors obtained medical records and pathology reports pertaining to removed lesions, and lesions were coded according to location, size and morphology.

Participants were eligible for this study if they had: (1) undergone a successful sigmoidoscopy with insertion to at least 50 cm with > 90% of mucosa visible or a suspect lesion identified; (2) completed the baseline risk factor questionnaire, and (3) provided a blood sample for use in etiologic studies. Of the 42,037 participants meeting these criteria, 4,834 were further excluded due to self-reported history of Crohn's disease, ulcerative colitis, familial polyposis, Gardner's syndrome, colorectal polyps, or cancer (other than non-melanoma skin cancer). We randomly selected 772 of the 1,234 cases with at least 1 distal (descending colon and sigmoid or rectum) advanced colorectal adenoma for genotyping. Advanced adenomas were those with at least 1 of the following 3 characteristics: (1) size of ≥ 1 cm; (2) high-grade dysplasia, or (3) villous components, including tubulovillous. Of the 26,651 controls with a negative sigmoidoscopy (no polyps or other suspect lesion detected), we selected 777 controls frequency-matched to cases by gender and ethnicity (non-Hispanic white, non-Hispanic black, and other). Participants with insufficient dietary data (missed 7 or more food items on the FFQ, n = 83) were further excluded, leaving a total of 720 advanced colorectal adenoma cases and 746 controls.

Gene Selection and Genotyping

All genes and SNPs were selected a priori based on known or suggested functional relevance and a minor allele frequency of ≥ 5% in Caucasians (Appendix 1). DNA was extracted from stored buffy coat or whole blood samples using Qiagen standard protocols (QIAamp DNA Blood Midi or Maxi kit; www1.qiagen.com). All genotyping was conducted at the Core Genotyping Facility of the Division of Cancer Epidemiology and Genetics, National Cancer Institute, using TaqMan (Applied Biosystems, Foster City, Calif., USA; www.appliedbiosystems.com). All of the assays were validated and optimized and methods specific to *GSTM1*, *GSTT1* and *GSTP1* have been reported elsewhere

[37]. Internal laboratory quality controls were Coriell DNA samples consisting of homozygous major allele, heterozygous and homozygous minor allele genotypes for each polymorphism under investigation. In every 384 samples, there were 4 of each control type and 4 no template controls. External blinded quality controls from 40 individuals were also interspersed and showed > 99% interassay concordance. Genotyping data were obtained for > 90% of subjects, with data missing for the following reasons: insufficient DNA, genotyping failures, or fingerprint profile review showing subject-specific ambiguities.

Dietary Data

Participants completed a 137-item FFQ with a detailed meat-cooking module on their usual diet during the previous year. Most (89%) participants in the trial completed the FFQ prior to or the same day as the sigmoidoscopy. Using the Computerized Heterocyclic Amines Resource for Research in Epidemiology of Disease (CHARRED; www.charred.cancer.gov) software application [4], we generated intake estimates of 2 HCAs (ng/day): 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), and 2-amino-1-methyl-6-phenyl-imidazo[4,5-*b*]pyridine (PhIP), as well as benzo[*a*]pyrene (B[*a*]P). We estimated nitrate and nitrite from processed meats using a nitrate/nitrite database based on laboratory measured values of these compounds from 10 types of processed meat samples that represented 90% of the processed meat consumed in the United States [4].

Statistical Analysis

We evaluated departure from Hardy-Weinberg equilibrium among the controls using Pearson's χ^2 tests. ORs and 95% CIs for the association between genotypes and advanced colorectal adenoma were calculated using unconditional logistic regression, adjusting for gender, ethnicity (non-Hispanic white, non-Hispanic black, other), and age (continuous). To evaluate the association between hypothesized gene pathways and colorectal adenoma, we included all the SNPs for genes potentially involved in the metabolism of each substrate in a model and compared it to a null model. We also conducted gene-specific global tests of association by including all of the SNPs in a given gene in a model and compared that to a null model that included none of the SNPs [38]. SNPs were coded with 2 dummy variables corresponding to the variant genotypes. The likelihood-ratio test for the gene-specific global test had 2*k* degrees of freedom (*k* representing the number of SNPs for the gene). Tests for linear trend were based on assigning ordinal values (0, 1 and 2) to the most prevalent genotypes in order of homozygous for the common allele, heterozygous and homozygous for the rare allele.

We evaluated effect modification of the associations between the meat-related exposure and colorectal adenoma by each of the XME genotype variants. We compared models with all of the cross product terms (diet on the continuous scale by genotype) to null models that included only the main effects. If this likelihood ratio test was statistically significant at the 0.05 level, we examined the effect of the dietary variable as a continuous measure stratified by genotype. Finally, to account for multiple comparisons, we corrected the p values for interactions using the False Discovery Rate [39].

Results

Cases and controls were similar with respect to the matching factors of gender and ethnicity (table 1). Cases tended to be older and were more likely to be current smokers and to have a first-degree relative with colorectal cancer. Cases also had fewer years of education and lower levels of physical activity compared to controls.

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Table 1. Baseline characteristics of subjects in a nested case-control study of advanced colorectal adenoma in the PLCO Cancer Screening Trial (n = 1,466).

Characteristics	Cases (n = 720)[a]	Controls (n = 746)[a]	p value[b]
Age, years	63.1 ± 5.2	61.9 ± 5.2	<0.01
Gender, n (%)			0.74
Male	501 (69.6)	513 (68.8)	
Female	219 (30.4)	233 (31.2)	
Ethnicity, n (%)			0.81
Non-Hispanic white	681 (94.6)	704 (94.4)	
Non-Hispanic black	15 (2.1)	19 (2.6)	
Other	24 (3.3)	23 (3.1)	
Study center, n (%)			<0.01
Colorado	65 (9.0)	84 (11.3)	
Georgetown	36 (5.0)	43 (5.8)	
Hawaii	14 (1.9)	13 (1.7)	
Henry Ford Health System	61 (8.5)	90 (12.1)	
Minnesota	136 (18.9)	173 (23.2)	
Washington	77 (10.7)	75 (10.1)	
Pittsburg	112 (15.6)	58 (7.8)	
Utah	62 (8.6)	38 (5.1)	
Marshfield	130 (18.1)	156 (20.9)	
Alabama	27 (3.8)	16 (2.1)	
First degree family history of colorectal cancer, n (%)	90 (12.5)	67 (9.0)	0.03
Education, n (%)			0.04
12 years or less	245 (34.0)	217 (29.1)	
At least some college	475 (66.0)	528 (70.9)	
Body mass index (kg/m^2)	27.9 ± 4.8	27.5 ± 4.6	0.09
Physical activity (h/week)	2.5 ± 1.8	2.8 ± 1.8	<0.01
Regular use of NSAIDs, n (%)	418 (58.1)	449 (60.2)	0.42
Smoking status, n (%)			<0.01
Never	243 (33.8)	300 (40.2)	
Former cigarette smoker	344 (47.8)	353 (47.3)	
Current cigarette smoker	98 (13.6)	50 (6.7)	
Never cigarettes, but pipe and cigar	34 (4.7)	43 (5.8)	
Alcohol (g/day)	14.5 ± 25.1	12.6 ± 24.0	0.27
Total caloric intake (kcal/day)	2,114 ± 834	2,168 ± 827	0.17
Red meat (g/day)	86.8 ± 64.3	87.7 ± 67.6	0.88
MeIQx (ng/day)	37.0 ± 51.8	35.8 ± 43.7	0.86
PhIP (ng/day)	203.1 ± 461.4	205.8 ± 458.6	0.83
B[*a*]P (ng/day)	30.7 ± 57.1	31.5 ± 53.3	0.92

Data are means ± standard deviations unless otherwise indicated.
NSAIDs = non-steroidal anti-inflammatory drugs.
[a] Numbers may not sum to total due to missing values
[b] p values are for χ^2 test for categorical variables and Wilcoxon rank sum test for continuous variables.

Table 2. Meat exposure XME gene pathways in relation to advanced colorectal adenoma

Meat exposure	Genes	p value[a]
HCAs	*CYP1A1, NAT1, NAT2, SULT1A1, SULT1A2*	0.312
PAHs	*CYP1A1, CYP1B1, CYP3A4, EPHX1, GSTM1, GSTP1, GSTT1, NQO1, SULT1A1, SULT1A2*	0.172
NOCs	*CYP2A6, CYP2C9, CYP2E1, GSTM1, GSTT1, GSTP1, NAT1, NAT2, NQO1*	0.225

[a] Global pathway test based on inclusion of all SNPs for a given pathway compared to a model without any SNPs.

Our investigation of XME pathways, in which we identified the multiple genes hypothesized to be involved in the metabolism of HCAs, PAHs and NOCs, yielded no statistically significant findings in relation to colorectal adenoma (table 2). Our results for *EPHX1, GSTM1, GSTM2, GSTT1, NAT1* and *NAT2* were similar to previously published results in the larger advanced colorectal adenoma PLCO case-control subset [37, 40–42] (data not shown). Expanding upon these earlier analyses, in our gene-specific global tests, we found associations for *GSTM1* (p value for global test = 0.03) and *NAT1* (p value for global test = 0.05) with advanced colorectal adenoma (data not shown). For individual genes and SNPs, we did not find any statistically significant associations between *CYP1A1, CYP1B1, CYP2A6, CYP2C9, CYP2E1, CYP3A4, NQO1*, *SULT1A1*, or *SULT1A2,* and advanced colorectal adenoma in this population (data not shown).

We found a suggestive interaction between intake of PhIP and variation in *CYP1B1* (rs10012 p for interaction = 0.019; rs1056836 p for interaction = 0.019) and *NQO1* (p for interaction = 0.007) with advanced colorectal adenoma (table 3). We also found evidence of interaction with intake of B[*a*]P for variation in *CYP1B1* (p for interaction = 0.005) and *CYP3A4* (p for interaction = 0.021). In addition, there was a possible interaction with intake of nitrate/nitrite and *CYP1A1* (p for interaction = 0.022). However, when we corrected for multiple comparisons, none of the resulting p values for interaction fell below a False Discovery Rate threshold of 0.20. When stratified by genotype, for *CYP1B1* rs10012, there was a statistically significant increased risk of colorectal adenoma with increasing intake of PhIP for participants with either the CG/GG genotypes (OR = 1.53; 95%CI = 1.02–2.30) and risk was also elevated among those with the CC genotype for *CYP1B1* rs1056836 (OR = 1.86; 95%CI = 1.07–3.22).

Discussion

Overall, we observed evidence of possible interactions between intake of meat-related HCAs, PAHs, and nitrate/nitrite and genetic variants in *CYP1A1*, *CYP1B1, CYP3A4,*

Table 3. ORs and 95% CIs for the association between dietary variables and advanced colorectal adenoma stratified by genotype.

Dietary intake	Gene	Locus	Genotype	Cases/ controls	OR[a]	$p_{interaction}$[b]	Corrected[c] $p_{interaction}$
PhIP per 1,000 ng/day	*CYP1B1*	rs10012	CC	344/375	0.81 (0.58–1.12)	0.019	0.384
			CG	293/296	1.47 (0.93–2.30)		
			GG	71/59	1.76 (0.60–5.21)		
			CG/GG	364/355	1.53 (1.02–2.30)		
	CYP1B1	rs1056836	CC	232/250	1.86 (1.07–3.22)	0.019	0.384
			CG	337/344	0.86 (0.64–1.15)		
			GG	137/137	0.82 (0.33–2.08)		
			CG/GG	474/481	0.85 (0.64–1.13)		
	NQO1	rs1800566	CC	416/474	1.03 (0.76–1.40)	0.007	0.340
			CT	244/225	0.99 (0.60–1.61)		
			TT	25/17	–		
			CT/TT	269/242	0.80 (0.52–1.24)		
B[*a*]P per 100 ng/day	*CYP1B1*	rs10012	CC	344/375	0.74 (0.55–1.00)	0.005	0.340
			CG	293/296	1.25 (0.94–1.68)		
			GG	71/59	1.66 (0.73–3.81)		
			CG/GG	364/355	1.29 (0.99–1.68)		
	CYP3A4	rs2242480	GG	558/579	1.02 (0.83–1.21)	0.021	0.384
			GA	126/131	1.13 (0.69–1.85)		
			AA	12/14	–		
			GA/AA	138/145	0.89 (0.56–1.42)		
Nitrate + Nitrite per 0.5 mg/day	*CYP1A1*	rs1048943	AA	646/684	1.03 (0.92–1.15)	0.022	0.384
			AG	44/38	1.11 (0.75–1.59)		
			GG	2/3	–		
			AG/GG	46/41	1.14 (0.79–1.64)		

Data are limited to SNPs with statistically significant tests for interaction before correction.
[a]Adjusted for age, gender and ethnicity.
[b]Likelihood ratio test for model with cross-product terms of dietary variables (coded as continuous) with the genotype variables (coded as dummy variables) compared to null model with only main effects for dietary variables and genotypes.
[c]Based on false discovery rate.

and *NQO1* with risk of advanced colorectal adenoma in the PLCO Cancer Screening Trial. Yet, when stratified by genotype, strong variation in risk of colorectal adenoma with increasing intake of the meat-related exposures was not obvious and correction for multiple comparisons indicated our findings may be due to chance. We did not observe any statistically significant main effects for *CYP1A1, CYP1B1, CYP2A6, CYP2C9, CYP2E1, CYP3A4, NQO1*, *SULT1A1* or *SULT1A2* on risk of advanced colorectal adenoma. Our gene-based analyses for *GSTM1* and *NAT1* support previously reported SNP based analyses in PLCO [37, 41].

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A strength of this analysis was our substrate-oriented pathway-based approach, in which we assessed a range of XME genes involved in the activation and detoxification of xenobiotics, and comprehensively examined interactions with meat-related intake of HCAs, PAHs and nitrate/nitrite. Our study was further strengthened by the inclusion of advanced colorectal adenoma cases, an outcome clinically relevant for progression to colorectal cancer. Importantly, since adenomas are largely asymptomatic, it is unlikely that cases would have changed their dietary habits. In addition, the majority of participants completed the FFQ prior to diagnosis, reducing the potential for recall bias. Our sample size is larger than many prior XME gene-meat interaction studies of colorectal adenoma and few have quantitatively estimated intake of the specific potentially carcinogenic meat-related exposures, instead relying on meat cooking method or doneness level as proxies.

A limitation of our analysis, like other studies of gene-environment interactions, is limited power to observe small associations and the potential for chance findings due to multiple comparisons. To gain power, we used a method for testing gene-environment interactions that assumes independence of the gene and the environmental factor [43], but in general, we did not observe smaller p values (data not shown). Future research of XME gene-meat interactions should assess both activating and detoxifying XME genes and evaluate the more specific meat-related exposures, rather than overall meat intake or meat cooking method/doneness. Yet these analyses can become complex, as there is a certain amount of error associated with the measurement of dietary exposures and their associated exposures. Finally, our measure of nitrate/nitrite is a proxy for processed meat-related exposure to NOCs and the nitrate/nitrite database does not contain data on the levels of these compounds in other foods.

In our interaction analyses, there was variation in the association between the meat-related variables and advanced colorectal adenoma across the *CYP1B1* genotypes. *CYP1B1* is involved in the metabolism of PAHs [14, 15, 44] and in fact, we did see a suggestive interaction with B[*a*]P, a known marker of PAHs [4]. Other studies have also found similar effect modification of the association between well-done red meat or total meat on colorectal cancer risk by combined *CYP1B1* variants [45, 46]. However, specific functional data for this variant and PAH metabolism are lacking and further work is required to characterize the biological mechanism underlying this potential interaction.

We found increased risk of colorectal adenoma with increasing PhIP intake among participants with the less common allele of *CYP1B1* rs10012 compared to the common allele and participants with the *CYP1B1* rs1056836 common allele (CC). Functionality of these variants in relation to PhIP is not well-characterized and, thus far, has been studied only in combination with other SNPs for this gene [47]. Another possible reason for an interaction between PhIP and the *CYP1B1* rs10012 variant is the relatively high correlation between PhIP and B[*a*]P (0.58) in our population.

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Variation in the association between our dietary variables and risk of colorectal adenoma by *CYP1A1*, *CYP3A4*, and *NQO1* was not straightforward. As hypothesized [48, 49], we did observe a suggestive interaction between *CYP3A4* variants and B[*a*]P intake on risk of advanced colorectal adenoma, but there have been no other studies of interaction with meat intake to verify this observation. *CYP3A4* is more common than other CYP3A isoforms in the intestine [50] and there is also wide range in expression levels of this enzyme across individuals [51], but little evidence as to which genetic variants control this variation [52]. One study of *CYP1A1* noted increased risk of colorectal adenoma among those with high meat intake [27]; however, 3 studies of colorectal cancer did not observe effect modification by meat [45, 53] or HCA intake [54]. One other study of colorectal cancer observed a possible interaction between *NQO1* phenotypes and red meat intake [55].

In general, there is little consensus in the literature for XME gene-meat interactions in relation to colorectal neoplasia for *CYP2A6* phenotypes [28, 56], *CYP2E1* [46, 57, 58], *EPHX1* [19, 25–27, 30, 55, 59], or S*ULT1A1* [20, 27, 45, 60–62]. In addition, there are limited data on *CYP2C9* [46] and *SULT1A2* [27]. Although we did not find evidence of effect modification by *NAT1* or *NAT2*, studies of these genotypes or phenotypes point toward an increased risk of colorectal neoplasia for rapid acetylators with high intake of meat, HCAs or PAHs [21, 22, 63–65]. Overall, these varied results could be due to several reasons, including differences in study populations and the study of adenomas versus cancer.

Our approach focused on a wide range of genes involved in the metabolism of 3 groups of potentially carcinogenic meat-related exposures: HCAs, PAHs, and nitrate/nitrite. Given our sample size, these analyses were largely exploratory. The substrate-focused pathway-based approach encompasses the multiple levels at which these potentially carcinogenic meat-related exposures are activated or detoxified in the body. With future consortial efforts, studies will have the opportunity to investigate potential effect modification of the association between meat-related exposures and colorectal adenoma by XME gene variants in greater detail.

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Appendix 1. XME genes included in main effect (pathway, gene and SNP) and/or interaction analyses

Gene	Locus
CYP1A1	Ex7+129C>A (T461N; rs1799814)
	Ex7+131A>G (I462V; rs1048943)[a]
CYP1B1	Ex2+143C>G (R48G; rs10012)
	Ex3+251G>C (V432L; rs1056836)
CYP2A6	Ex3-15T>A (L160H; rs1801272)
CYP2C9	Ex3-52C>T (R144C; rs1799853)
CYP2E1	-332T>A (rs2070673)
	IVS4+23T>C (rs6413421)
CYP3A4	IVS10+12G>A (rs2242480)
EPHX1[b]	Ex3-28T>C (Y113H; rs1051740)
	Ex4+52A>G (H139R; rs2234922)
GSTM1[c]	Ex4+10+>- (rs1065411)
GSTP1[c]	Ex5-24A>G (I105V; rs1695)
	Ex17-4C>T (H1085H; rs1799817)
GSTT1[c]	Ex5-49+>- (rs4630)
NAT1[a]	Ex3-177A>T (T1088A; rs1057126)
	Ex3-170A>C (C1095A; rs15561)
	IVS2-338C>T (C-334T; rs4986988)
	IVS2-34A>T (A-40T; rs4986989)
NAT2[a]	Ex2-367G>A (R268K; rs1208)
	Ex2-313G>A (G286E; rs1799931)
	Ex2+288C>T (Y94Y; rs1041983)
	Ex2+347T>C (I114T; rs1801280)
	Ex2+487C>T (L161L; rs1799929)
	Ex2-580G>A (R197Q; rs1799930)
NQO1	Ex4-3C>T (R139W; rs4986998)
	Ex6+40C>T (P187S; rs1800566)[a]
SULT1A1	Ex10+127A>G (G212G; rs6839)
SULT1A2	336bp 3′ of STP (rs3194168)

[a] SNP main effects previously published for advanced colorectal adenoma in the PLCO Cancer Screening Trial.
[b] SNP main effects and interactions with red meat and dietary B[*a*]P previously published for advanced colorectal adenoma in the PLCO Cancer Screening Trial.
[c] SNP main effects and interactions with red meat, HCAs and B[*a*]P previously published for advanced colorectal adenoma in the PLCO Cancer Screening Trial.

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