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Colorectal polyp type and the association with charred meat consumption, smoking, and microsomal epoxide hydrolase polymorphisms

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Abstract

Objective—We determined the association between charred meat consumption, cigarette smoking, microsomal epoxide hydrolase (*mEH*) polymorphisms [rs1051740 and rs2234922], and colorectal adenomas and hyperplastic polyps (HPs) and explored gene-environment interactions.

Methods—Men and women with colorectal adenomas (n=519), HPs (n=691), or concurrently with both types of polyps (n=227) and polyp-free controls (n=772) receiving a colonoscopy from 12/04–9/07 were recruited. Participants completed telephone interviews and provided buccal cell samples; genotyping of *mEH* was completed using Taqman assays. We conducted polytomous regression and calculated odd ratios (OR) and 95% confidence intervals. Interactions were evaluated using Wald chi-square tests.

Results—Consumption of >3 servings of charred meat per week was associated with distal HPs (OR=2.0, 1.2–3.4) but not adenomas nor either type of proximal polyp. Heavy cigarette smoking (≥22 pack-years) was associated with an increased risk for colorectal adenomas (OR=1.7, 95% CI 1.2–2.4), HPs (OR=2.4, 95% CI 1.7–3.3), and both types (OR=2.8, 95% CI 1.8–4.3) with the strongest association for distal polyps. There was no association between *mEH* genotype and colorectal polyps, nor were any statistically significant gene-environment interactions identified.

Discussion—Future investigation of BaP exposure and colorectal neoplasia should analyze whether associations are dependent upon anatomic location.

Introduction

Several studies report that consumption of charred red meat is a risk factor for certain colorectal polyps, including adenomas (1–7), established precursor lesions for colorectal cancer (8), and possibly, hyperplastic polyps (HPs) (6), common polyps recently hypothesized to represent a separate pathway to colorectal cancer (9–11). Cigarette smoking is also associated with an increased risk of adenomas (12–18) and HPs (13–16).

Both cigarette smoking and charred meat consumption result in exposure to benzo[a]pyrene (BaP), a polycyclic aromatic hydrocarbon (PAH) that is chemically inert in the environment

(19). However, after BaP enters the body, it is metabolized into benzo[a]pyrene diol-epoxide (BPDE), a strong carcinogen that can form DNA adducts (20, 21). Therefore, cigarette smoking and charred meat consumption may share a mechanism for carcinogenesis that involves ingestion and metabolism of BaP.

Microsomal epoxide hydrolase (mEH) is an enzyme that detoxifies a number of environmental compounds once they enter the body (22). However, in the case of BaP, mEH results in the metabolic conversion of BaP to BPDE, thereby potentially increasing the risk of DNA adducts, mutation, and subsequent cancer initiation (20, 23). As BaP enters the body, it is activated by several enzymes; CYP1A1 or CYP1B1 metabolizes BaP into an epoxide. Then, mEH hydrolyzes the epoxide to a dihydrodiol, and CYP1A1, CYP1B1, or CYP3A4 subsequently transforms the dihydrodiol to BPDE (20).

Two functional polymorphisms in the *mEH* gene have been well-characterized. One, in exon 3 of the gene (rs1051740), results in a Tyr113His substitution and a 40–50% decrease in mEH activity *in vitro* (24). The other polymorphism (rs2234922) occurs in exon 4 causing a His139Arg substitution and a 25% increase in mEH activity (24).

Because mEH is important in the activation of BaP, and due to the associations between colorectal neoplasia and exposures resulting in ingestion of BaP (*i.e.* smoking and charred meat consumption), several studies have examined the relationship between *mEH* polymorphisms and colorectal neoplasia. The results for colorectal cancer (25–28) and polyp risk (27, 29–34) have been mixed, but it may be that variation in mEH activity is important only among those with high exposure to BaP.

In this study, we evaluated the association between charred meat consumption, cigarette smoking, *mEH* polymorphisms, and the risk of colorectal adenomas and HPs, as well as potential gene-environment interactions. By examining mEH activity in the context of risk factors that increase exposure to BaP, we sought to better understand the biological mechanisms by which the common exposures of charred meat consumption and cigarette smoking promote carcinogenesis in the colorectum. In addition, we looked to provide one of the answers to the pragmatic question of why some people are more susceptible to the carcinogenic effects of charred meat consumption and cigarette smoking than others.

Methods

Study Population

This study was conducted among enrollees of Group Health, a large integrated-health plan in Washington state, aged 20–74 years who underwent colonoscopy for any indication, between December 2004 and September 2007. This exam was considered their “index” colonoscopy. Eligible cases were patients diagnosed with adenomatous and/or HPs of the colorectum at this examination. Control subjects had normal findings at their index exam (*i.e.*, no biopsies were performed, and no colorectal disease was detected).

Participants were ineligible for this study if, based on review of electronic medical records, they had a colonoscopy within the 12 months prior to their index colonoscopy; they were enrolled in Group Health for fewer than 3 years (and thus their medical record may be incomplete); or if they had a prior diagnosis of colorectal cancer, inflammatory bowel disease, familial adenomatous polyposis, or Lynch Syndrome. Participants were also excluded if their index examination was incomplete based on one or more of the following findings: the cecum was not visualized at endoscopy; surgical excision of lesions found at index examination was recommended; and/or bowel preparation was considered inadequate.

Case subjects were classified on the basis of type and location of polyps found at colonoscopy. For this investigation, cases were categorized as having: 1) colorectal adenomas (ICD9 211.3); 2) HPs (ICD9 211.4); or 3) both, the presence of both a colorectal adenoma and hyperplastic polyp at index exam (ICD9 211.3 and 211.4). Eligible controls were selected randomly within five-year age categories and calendar year of colonoscopy. Study procedures were approved by the Institutional Review Boards of Group Health and the Fred Hutchinson Cancer Research Center.

Data Collection

After colonoscopy, potential study participants were contacted via telephone, explained the study purpose and procedures, and gave oral consent to participate in a 45-minute telephone interview. This standardized interview covered the following information: patient demographics, meat consumption and preparation, smoking history, weight and height, lifetime alcohol consumption, medication use, medical history, and family history of colorectal cancer. For women, reproductive history and hormone use was also collected. The majority of study participants were interviewed within 3–4 months of their index colonoscopy. A reference date was established as one year prior to the index colonoscopy date, and subjects reported exposures, such as cigarette smoking, which occurred prior to the reference date.

After the completion of the interview, participants were mailed forms and gave written consent for medical records review and release of diagnostic materials. Further, participants were asked to provide a buccal sample for DNA testing. Those who agreed to provide a sample were mailed a buccal cell mouthwash kit, which included detailed instructions for obtaining the buccal sample, based on the protocol by Lum, et al (35). The participation rate for the telephone interview was approximately 75%, resulting in 2209 eligible study participants. Participation rates were similar across diagnosis groups. Among those interviewed, buccal samples were obtained from approximately 76% of polyp cases and 90% of control participants.

Genotyping

Single nucleotide polymorphism (SNPs) in *mEH* were selected based on their reported functional activity (24).

Genomic DNA was extracted from buccal samples using Qiagen's QIAmp DNA extraction kit (Qiagen, Valencia, CA) and quantified by Picogreen (Invitrogen, Carlsbad, CA). DNA samples were genotyped using ABI pre-developed Taqman Allelic Discrimination Assays (Applied Biosystems, Foster City, CA). Briefly, PCR was performed in 384 well plates with 10ng DNA, 2.5 ul 2X TaqMan Master Mix (Applied Biosystems), 0.25 ul 20x assay mix in a total volume of 5 ul. Cycling conditions were: 50°C for 2 min, 95°C for 10 min, 40 cycles of 92°C for 15 sec, 60°C for 1min. Plates were read on an ABI 7900HT sequence detection system using SDS 2.3 software. Each plate included positive controls of known genotype obtained from the Coriell Biorepository (Rutgers, NJ) and negative controls with no DNA added. Assay accuracy was verified by comparing genotypes generated for a panel of Coriell DNA samples to publicly available genotype data for these samples from HapMap (<http://www.hapmap.org/>). In addition, 62 samples (3.5%) which were included as duplicates in the study gave 100% concordant genotypes.

Of the 1765 buccal DNA samples tested, 1677 (95%) were genotyped successfully for both polymorphisms. The polymorphisms in these analyses were in Hardy-Weinberg equilibrium in Caucasian controls (n=515) (36).

Statistical Analysis

We performed multivariable polytomous regression analyses to estimate the relative risk and 95% confidence intervals (CI) of colorectal adenomas only, HPs only, and both lesions concurrently, relative to controls utilizing SAS version 9.1 (SAS Institute, Inc, Carey, NC).

Exposure to charred red meat (beef, veal, lamb, mutton, pork, or venison cooked by pan-frying, broiling, grilling, or barbecuing) was analyzed according to the preparation of the meat (lightly browned, medium browned, heavily browned/blackened) and number of servings per week (0, 1–3, and >3 servings per week), where one serving was defined as 2–3 ounces of meat, or “about the size of a deck of cards”. Cigarette smoking status was categorized as never, former, or current at the time of the reference date. We also estimated the life-time dose of cigarette smoking by calculating pack-years for each study participant; one pack-year was defined as 20 cigarettes per day for one year.

Additionally, we assigned each participant a predicted mEH activity level (slow, medium, fast) according to the combination of *mEH* polymorphisms in exons 3 and 4 using the classification by Benhamou, et al (37), as evaluated by Zhang, et al (38) and Viezzer, et al (39).

Statistical models assessing the association between polyps, smoking, and charred meat consumption included the following potential confounders, chosen *a priori* based on the literature: age, gender, race, education, body mass index (BMI), alcohol intake (drinks per week), non-steroidal anti-inflammatory drug (NSAID) use, and hormone therapy (women only). Smoking and charred meat intake models were also adjusted for one another by including both of these variables in the same model. Statistical models evaluating the association between *mEH* polymorphisms and each case group were adjusted for age, gender, and race as were models with predicted mEH activity.

To explore whether the association between our exposures of interest and each polyp type were dependent upon the location of polyps, we conducted polytomous regression analyses similar to those describe above but restricting cases to the following groups: exclusively proximal adenomas, exclusively distal and/or rectal adenomas, exclusively proximal HPs, and exclusively distal and/or rectal HPs. Polyp location was determined via medical record abstraction.

To evaluate gene-environment interactions, we stratified our *mEH* analyses by levels of smoking and charred red meat intake. In addition, we conducted Wald chi-square tests for interactions using separate logistic regression models that included the cross-product term between the genotype (or predicted mEH activity level) and the risk factor of interest (*i.e.* smoking or charred red meat consumption), adjusting for age, sex, and race for each case group.

Results

Over half of participants were 60 years and older (Table 1). Adenoma cases, and cases with both types of polyps, were more likely than controls to be male, have a BMI ≥ 30 , consume more than 7 alcoholic beverages per week, and were less likely to use NSAIDs or hormone therapy (women only). Hyperplastic polyp cases were also more likely than controls to be male, consume >7 alcoholic beverages per week, and were less likely to use NSAIDs; however, they were similar to controls with respect to BMI and hormone therapy use (Table 1).

When location of the each polyp type was not considered, consumption of charred red meat was suggestively associated with elevated risks in each case group; however, the associations were not significant (Table 2). Cigarette smoking was strongly associated with increased risk of all polyp types. The strongest associations were observed in cases with HPs and in participants concurrently diagnosed with both types of polyps. For adenomas, smoking ≥ 22 pack-years was associated with a 1.7-fold increased risk (95% CI: 1.15 to 2.37), and being a current smoker was associated with an elevated, but not significant, risk [OR (95% CI): 1.62 (0.94 to 2.33)]. For HPs, the estimated odds ratio (OR) for smoking ≥ 22 pack-years was 2.36 (1.69 to 3.30) and for current smoking status, it was 2.87 (1.80 to 4.58). For participants concurrently diagnosed with both types of polyps, these estimates were 2.80 (1.82 to 4.32) and 3.90 (2.12 to 6.93), respectively (Table 2). Neither the polymorphisms in *mEH* nor predicted mEH activity were significantly associated with any case group (Table 2).

In the analyses comparing cases with proximal polyps to those with distal and/or rectal polyps, there is clearly no association between charred meat consumption/preparation or cigarette smoking history and proximal polyps for either polyp group (Table 3). However, charred meat consumption was associated with a significant 2-fold increase in the risk of distal and/or rectal HPs (95% CI: 1.20 to 3.44 for greater than 3 servings per week) and an insignificant 50% increase in the risk of proximal and/or rectal adenomas (95% CI: 0.87 to 2.56) (Table 3). Cigarette smoking was associated with significantly increased risks of distal and/or rectal polyps for both polyp types (Table 3).

Stratified analyses and Wald chi-square tests (40) revealed no evidence for significant interactions between *mEH* genotypes and pack-years of smoking (interaction p-values ranged from 0.31 to 0.92), nor between *mEH* genotypes and servings of charred red meat (interaction p-values ranged from 0.40 to 0.97) for any case group (data not shown). In addition, analyses of predicted mEH activity, based on a combination of the two polymorphisms, indicated no significant interactions (Table 4).

Discussion

Results from our study support previous investigations demonstrating a positive association between cigarette smoking and colorectal polyps (12–18). Furthermore, we confirmed results from studies done in other populations reporting that adenomas are associated with smoking separate from HPs (14–16). Also, like these prior studies, we reported that cigarette smoking is more strongly associated with HPs than adenomas. For adenomas, previous studies reported ORs ranging from 1.3–1.6 associated with the highest level of smoking, and for HPs, the OR estimates ranged from 3.1 to 4.8 [5–7]. Based on these findings, we concur with Morimoto, *et al* (14) that cases having both adenomas and HPs should be evaluated separately from those with only adenomas or only HPs. These analyses are in contrast to studies that include individuals with both adenomas and HPs in the adenoma case group (41, 42). In the instance of smoking, including those with both types of polyps in the adenoma case group, rather than putting them in their own separate group, would result in an inflation of the OR estimate for adenomas.

With the growing body of evidence that a subset of HPs may progress to cancer along a pathway that is distinct from the established adenoma-carcinoma sequence (43), it is important to characterize risk factors for adenomas and HPs separately from one another, and to consider those with both types of polyps as an independent case group. However, cases with both types of polyps were different from the other case groups in that they necessarily had more than one polyp. When we restricted our analyses to adenoma only cases and HP only cases with multiple polyps, the OR estimate for smoking ≥ 22 pack-years

increased by 8% for adenomas and by 32% for HPs (data not shown). This suggests that the stronger association noted in those with both polyp types may reflect the fact that these cases have multiple polyps, one of which is a hyperplastic polyp.

Our study results also suggest a positive association between charred meat consumption and distal/rectal HPs. We did not report statistically significant associations between charred red meat consumption or preparation and adenomas, in contrast to several such studies(1–7). Risk estimates evaluating the association between servings of charred red meat per week and colorectal adenomas for these studies range between 1.11 and 2.73. Despite finding no statistically significant associations for charred red meat intake and adenomas, we reported a suggestion of elevated risks associated with consuming charred red meat, and these risk estimates were higher for adenomas in the distal colon/rectum.

Numerous studies have reported that the increased risk of colorectal polyps associated with smoking is exclusively in distal and rectal polyps; however, this has not been well-studied for meat intake, and further research is needed to determine if the association between charred meat consumption and colorectal polyps depends on polyp location. This information could provide clues to the mechanisms by which charred meat may promote polyp growth and contribute to increased colorectal cancer risk.

Our results do not support a strong association between *mEH* polymorphisms and colorectal polyp risk. Other studies have evaluated the association between *mEH* polymorphisms and colorectal polyps, and the results are inconsistent. Two studies reported significantly decreased risks of adenomas, ranging from a 30–44% reduction in risk, associated with the exon 3 His/His genotype (slower *mEH* activity) (27, 29). Five additional studies reported no association for the exon 3 polymorphism with adenomas (30–34), and two of these also evaluated HPs and reported no association for HPs (31, 34). Each of the above studies evaluated the exon 4 polymorphism in relation to polyp risk, and only one reported a significant increased risk for adenomas (OR= 1.72) associated with the Arg/Arg genotype (faster *mEH* activity) (33).

Of note is that the three studies reporting significant associations between colorectal polyps and *mEH* polymorphisms all evaluated adenomas and not HPs, had large sample sizes (ranging from 772–991 cases and from 400–946 controls), and identified cases via sigmoidoscopy rather than colonoscopy (27, 29, 33). Therefore, their case groups all have either rectal or distal colon polyps. It is possible that variations in *mEH* activity play a role in rectal and distal colonic neoplasia but not in other parts of the large bowel.

We attempted to explore this possibility in our data by evaluating genotype associations among cases with at least one left-sided or rectal polyp (data not shown). No significant associations were identified for the exon 3 polymorphism when we restricted our analyses to cases with left-sided or rectal polyps. However, for the Exon 4 polymorphism, the homozygous variant allele was associated with a statistically significant increase in the risk of being diagnosed with both types of polyps concurrently when at least one of these polyps was in the left colon or rectum [OR (95% CI): 2.48 (1.02–6.04)].

Consistent with our results, several studies examining an interaction between *mEH* genotype or predicted *mEH* activity and cigarette smoking (29–34) or consumption of charred meat (30–32, 34) did not find any gene-environment interactions. A subset of these studies examined charred meat intake as a potential modifier of the association between *mEH* polymorphisms and colorectal neoplasia and reported results that were similar to smoking analyses, with the exception that one study reported evidence for a statistically significant interaction between predicted *mEH* activity and charred meat consumption (interaction p-

value = 0.03) (32). Among those with high charred meat consumption, Cortessis et al. (32) found “rapid” *mEH* activity increased the risk of adenomas.

The present study has several strengths, including a well-characterized population of comparable cases and controls and standardized assessment of epidemiologic risk factors. In addition, since all cases and controls were evaluated via colonoscopy, we ensured that controls were, in fact, polyp-free. Finally, we evaluated functional polymorphisms with known effects on *mEH* activity, allowing us to examine a potential mechanism for smoking and charred meat induced neoplasia in the colon and rectum.

Despite these strengths, our findings should be interpreted in light of several limitations. We interviewed cases and controls after diagnosis, which may result in differential recall bias (44). However, because polyps tend to be presented to patients as benign, case status is unlikely to greatly influence responses. Also, genotype was not known to study participants. Because we selected study participants who received a colonoscopy, our study population was likely healthier (45), with less heavy smokers, and likely consumed less red meat than the greater Group Health population. This would potentially reduce our power to detect significant associations due to a lower prevalence of these exposures in our study population.

With the addition of the present study, eight studies have examined the role of *mEH* polymorphisms and colorectal polyp risk. However, there is still little consensus on the role that variations in *mEH* activity play in colorectal carcinogenesis, either directly or as a co-factor with environmental exposures to BaP. The only studies that report significant associations between *mEH* polymorphisms and colorectal polyps focused on the distal colon and rectum and had large samples sizes. In addition, in our own analyses, the associations between polyps and smoking and charred meat consumption were stronger in the distal colon/rectum.

Therefore, we recommend that future studies of mechanisms for BaP induced colorectal carcinogenesis recruit enough participants with left-sided and rectal tumors that stratified analyses by site can be conducted with adequate power. Also, because clinic-based colonoscopy or sigmoidoscopy studies may have lower levels of heavy smoking and charred meat consumption, future studies need to allow for larger sample sizes to adequately address questions on gene-environment interactions.

Finally, given the lack of strong evidence for the role of *mEH* in colorectal carcinogenesis, other potential mechanisms for BaP associated colorectal neoplasia need to be fully evaluated. One such mechanism involves one-electron oxidation of BaP, producing radical cations and resulting in depurinating DNA adducts (46, 47). These adducts are unstable, and they break off from the DNA (48), leaving apurinic sites repaired by error-prone base-excision repair (49). For this reason, BaP’s association with unstable DNA adducts may be a much more important mechanism for carcinogenesis than the formation of stable adducts, like BPDE. Further research is required to determine mechanisms for BaP-related carcinogenesis and potential genetic effect modifiers of this pathway.

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Table 1Characteristics of study participants by colorectal polyp status (N=2209^a), Group Health.

	Controls (n=772)	Adenomas (n=519)	Hyperplastic Polyps (n=691)	Both Types of Polyps (n=227)
	N (%)	N (%)	N (%)	N (%)
Age				
24–49	50 (6)	29 (6)	45 (7)	12 (5)
50–59	318 (41)	203 (39)	329 (48)	89 (39)
60–69	296 (38)	205 (39)	233 (34)	88 (39)
70–74	108 (14)	82 (16)	84 (12)	38 (17)
Male	309 (40)	259 (50)	304 (44)	133 (59)
Caucasian	661 (86)	431 (83)	592 (86)	193 (85)
Education				
Less Than High School	10 (1)	9 (2)	5 (1)	1 (<1)
High School Graduate or GED	84 (11)	46 (9)	47 (7)	21 (9)
Some College/Vocational School	214 (28)	147 (28)	222 (32)	86 (38)
College Graduate	464 (60)	317 (61)	416 (60)	116 (52)
BMI				
<25	319 (41)	170 (33)	277 (40)	68 (30)
25–29.9	293 (38)	202 (39)	263 (38)	82 (36)
≥30	158 (21)	143 (28)	148 (22)	76 (34)
Alcohol intake (drinks/wk)				
0	374 (48)	247 (48)	332 (48)	97 (43)
1–7	240 (31)	132 (25)	195 (28)	61 (27)
>7	158 (21)	140 (27)	164 (24)	69 (30)
NSAIDS use (Yes)	534 (69)	345 (66)	446 (65)	143 (63)
Hormone therapy use^b				
Never	214 (46)	117 (45)	193 (50)	47 (50)
Former	179 (39)	116 (45)	138 (36)	37 (39)
Current	68 (15)	27 (10)	55 (14)	10 (11)

^aDue to missing values, totals do not always sum to 2209^bAmong women only

Adjusted odds ratios (ORs) for colorectal polyp types in relation to cigarette smoking, red meat consumption and preparation, and mEH polymorphisms.

Table 2

	Controls			Adenomas			Hyperplastic Polyps			Both Types of Polyps		
	N	N	OR	N	OR	95% CI	N	OR	95% CI	N	OR	95% CI
Servings red meat/wk^a												
0 (ref)	101	54	1.00				69	1.00		23	1.00	
1-3	415	281	1.25			0.86-1.82	383	1.41		118	1.49	0.85-2.35
>3	224	181	1.19			0.80-1.78	231	1.34		83	1.31	0.73-2.35
p-trend			0.58					0.32			0.70	
Red meat prep^a												
No red meat (ref)	101	54	1.00				69	1.00		23	1.00	
Lightly browned	89	67	1.38			0.87-2.19	91	1.42		32	1.62	0.85-3.10
Medium browned	400	273	1.28			0.89-1.84	367	1.36		117	1.42	0.84-2.43
Heavily browned/Blackened	169	121	1.35			0.89-2.02	156	1.34		52	1.44	0.80-2.59
p-trend			0.38					0.21			0.76	
Smoking pack-yrs^a												
0 (ref)	459	277	1.00				353	1.00		105	1.00	
0.01-21.99	225	151	1.10			0.85-1.43	210	1.28		61	1.10	0.76-1.59
≥22	81	84	1.65			1.15-2.37	123	2.36		58	2.80	1.82-4.32
p-trend			0.02					<0.01			<0.01	
Smoking status^a												
Never (ref)	459	277	1.00				353	1.00		105	1.00	
Former	280	211	1.19			0.93-1.53	274	1.38		90	1.24	0.88-1.75
Current	32	31	1.62			0.94-2.77	64	2.87		32	3.90	2.19-6.93
p-trend			0.05					<0.01			<0.01	
mEH Exon 3^b												
Tyr/Tyr (Fast) (ref)	281	201	1.00				242	1.00		77	1.00	
His/Tyr (Medium)	252	163	0.91			0.70-1.19	225	1.03		82	1.19	0.83-1.70
His/His (Slow)	56	33	0.83			0.52-1.32	51	1.05		14	0.89	0.47-1.69
p-trend			0.30					0.39			0.78	

	Controls		Adenomas			Hyperplastic Polyps			Both Types of Polyps		
	N		N	OR	95% CI	N	OR	95% CI	N	OR	95% CI
mEH Exon 4^b											
His/His (Slow) (ref)	379		248	1.00		323	1.00		102	1.00	
Arg/His (Medium)	193		130	1.02	0.77–1.34	173	1.04	0.81–1.34	63	1.19	0.83–1.71
Arg/Arg (Fast)	17		19	1.77	0.90–3.49	22	1.55	0.81–2.98	8	1.85	0.77–4.43
p-trend				0.29			0.30			0.13	
Predicted mEH activity^{b,c}											
Slow (ref)	224		138	1.00		192	1.00		64	1.00	
Medium	247		172	1.14	0.86–1.53	222	1.05	0.80–1.37	71	1.02	0.69–1.50
Fast	118		87	1.21	0.85–1.71	104	1.03	0.75–1.44	38	1.13	0.71–1.80
p-trend				0.26			0.80			0.62	

^a Adjusted for age, gender, race, education, BMI, alcohol intake, NSAIDs use, and hormone therapy use. Additionally, meat analyses are adjusted for smoking pack-years and smoking analyses adjusted for servings of fried, broiled, or grilled red meat per week (N=2209)

^b Adjusted for age, sex, race (N=1677)

^c Predicted mEH activity is based on the classification by Benhamou, et al. (36)

Table 3

Adjusted odds ratios (ORs) for adenomas and hyperplastic polyps in relation to red meat consumption and preparation, cigarette smoking, and mEH polymorphisms split by anatomic location.

	Adenomas						Hyperplastic Polyps						
	Controls			Distal/Rectal (N=187)			Proximal (N=235)			Distal/Rectal (N=339)			
	N	OR	95% CI	N	OR	95% CI	N	OR	95% CI	N	OR	95% CI	
Servings red meat/wk ^a													
0 (ref)	101	21	1.00	24	1.00		31	1.00		26	1.00		
1-3	415	101	1.10	0.65-1.88	142	1.58	0.95-2.63	128	1.01	0.64-1.59	189	2.07	1.26-3.42
>3	224	63	1.10	0.62-1.94	120	1.49	0.87-2.56	73	1.01	0.61-1.66	120	2.03	1.20-3.44
p-trend			0.82			0.32			0.97			0.05	
Red meat prep ^a													
No red meat (ref)	101	21	1.00	24	1.00		31	1.00		26	1.00		
Lightly browned	89	26	1.27	0.66-2.45	29	1.37	0.72-2.59	32	1.15	0.64-2.06	41	1.86	1.01-3.42
Medium browned	400	90	0.99	0.58-1.66	143	1.59	0.96-2.66	124	1.02	0.64-1.62	187	2.10	1.27-3.47
Heavily browned/Blackened	169	48	1.28	0.71-2.30	59	1.55	0.89-2.73	45	0.90	0.53-1.53	81	2.08	1.21-3.59
p-trend			0.61			0.11			0.58			0.01	
Smoking pack-yrs ^a													
0 (ref)	459	104	1.00			1.00		154	1.00		145	1.00	
0.01-21.99	225	75	1.11	0.76-1.61	39	1.06	0.75-1.48	62	0.88	0.62-1.24	113	1.66	1.22-2.26
≥22	81	8	1.23	0.72-2.12	70	2.02	1.30-3.14	18	0.86	0.49-1.52	78	3.70	2.49-5.50
p-trend			0.41		45	0.01			0.44			<0.01	
Smoking status ^a													
Never (ref)	459	68	1.00		139	1.00		154	1.00		145	1.00	
Former	280	55	1.14	0.81-1.61	101	1.23	0.93-1.64	72	0.87	0.63-1.21	154	1.87	1.40-2.50
Current	32	16	1.22	0.53-2.80	16	1.77	0.92-3.42	9	0.87	0.38-1.99	40	4.21	2.47-7.15
p-trend			0.43			0.06			0.42			<0.01	
mEH Exon 3 ^a													
Tyr/Tyr (Fast) (ref)	281	90	1.00		102	1.00		93	1.00		110	1.00	

Adenomas														Hyperplastic Polyps					
Controls		Proximal (N=187)			Distal/Rectal (N=256)			Proximal (N=235)			Distal/Rectal (N=339)								
		N	OR	95% CI	N	OR	95% CI	N	OR	95% CI	N	OR	95% CI						
His/Tyr (Medium)		252	42	0.89	0.60–1.32	88	0.95	0.68–1.32	70	0.84	0.59–1.32	114	1.15	0.84–1.58					
His/His (Slow)		56	7	1.16	0.63–2.16	13	0.62	0.32–1.18	18	1.01	0.63–2.16	29	1.33	0.81–2.20					
p-trend				0.62			0.23			0.95			0.21						
<i>mEH Exon 4^c</i>																			
His/His (Slow) (ref)		379	54	1.00		123	1.00		117	1.00		157	1.00						
Arg/His (Medium)		193	57	0.92	0.61–1.38	70	1.12	0.79–1.57	53	0.88	0.61–1.27	89	1.12	0.82–1.53					
Arg/Arg (Fast)		17	28	1.67	0.67–4.16	5	1.97	0.87–4.45	11	2.23	1.01–4.93	7	1.06	0.43–2.61					
p-trend				0.30			0.17			0.65			0.63						
Predicted mEH activity^{a,b}																			
Slow (ref)		224	54	1.00		68	1.00		63	1.00		101	1.00						
Medium		247	57	0.97	0.64–1.47	87	1.18	0.82–1.71	79	1.13	0.78–1.66	105	0.94	0.67–1.31					
Fast		118	28	0.99	0.59–1.64	48	1.38	0.89–2.13	39	1.16	0.74–1.84	47	0.90	0.59–1.36					
p-trend				0.93			0.15			0.71			0.60						

^a Adjusted for age, gender, race, education, BMI, alcohol intake, NSAIDs use, and hormone therapy use. Additionally, meat analyses

^b Adjusted for age, sex, race (N=1677)

^c Predicted mEH activity is based on the classification by Benhamou, et al. (36)

Table 4

Adjusted^a polytomous regression analyses of the association between predicted mEH activity^b and colorectal polyps stratified by levels of smoking and charred red meat intake (N=1677).

Adenomas										Hyperplastic Polyps						Both Types of Polyps					
Servings of charred red meat/week										Servings of charred red meat/week						Servings of charred red meat/week					
0		1-3		>3		0		1-3		>3		0		1-3		>3					
OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI				
Predicted mEH activity ^b																					
Slow (ref)	1.00			1.00			1.00			1.00			1.00			1.00					
Medium	1.15	0.48-2.73	1.17	0.83-1.66	1.15	0.58-2.32	1.19	0.52-2.73	1.03	0.75-1.40	1.09	0.55-2.17	1.95	0.45-8.53	0.84	0.53-1.33	1.73	0.70-4.25			
Fast	1.06	0.35-3.20	1.30	0.85-1.99	0.88	0.40-1.93	1.58	0.59-4.21	1.04	0.71-1.54	0.77	0.35-1.68	4.20	0.91-19.39	0.77	0.43-1.39	1.65	0.63-4.33			
Interaction p-value		0.53						0.35						0.81							
Pack-years smoking										Pack-years smoking						Pack-years Smoking					
0		0.01-21.99		≥22		0		0.01-21.99		≥22		0		0.01-21.99		≥22					
OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI				
Predicted mEH activity ^b																					
Slow (ref)	1.00			1.00			1.00			1.00			1.00			1.00					
Medium	1.16	0.86-1.53	1.37	0.81-2.34	0.73	0.34-1.60	0.97	0.68-1.38	1.23	0.75-2.03	0.83	0.41-1.73	0.99	0.59-1.67	0.84	0.37-1.93	0.94	0.39-2.28			
Fast	1.21	0.85-1.71	0.74	0.39-1.40	2.42	0.77-7.59	1.24	0.80-1.90	0.78	0.43-1.40	1.28	0.40-4.04	0.82	0.41-1.64	1.21	0.53-2.77	2.92	0.85-10.09			
Interaction p-value		0.65						0.57						0.13							

^a Adjusted for age, sex, race (N=1677)

^b Predicted mEH activity is based on the classification by Benhamou, et al. (36)