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Advances in Brief

A Case-Control Study of Microsomal Epoxide Hydrolase, Smoking, Meat Consumption, Glutathione *S*-Transferase M3, and Risk of Colorectal Adenomas[1]

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Abstract

We estimated associations between polymorphisms in the gene encoding microsomal epoxide hydrolase (mEH) among 464 cases diagnosed with first occurrence of colorectal adenoma and 510 matched controls. In an analysis controlling only for the matching variables, we found little or no association between adenoma and *mEH* genotypes defined by polymorphisms at either codon 113 and 139 or mEH activity predicted by both polymorphisms. However, in subsequent analyses, high predicted mEH activity was significantly associated with adenoma among certain subgroups defined by smoking history [odds ratio (OR), 4.27; 95% confidence interval (CI), 1.68–10.81 among current smokers; interaction, $P = 0.11$], meat consumption (OR, 2.47; CI, 0.99–6.19 among individuals who regularly eat well-done meat; interaction, $P = 0.03$), and genotypes for the **A/*B* polymorphism in the gene encoding glutathione *S*-transferase M3 (OR, 2.60; CI, 1.28–5.28 among individuals with **A*A* genotype; interaction, $P = 0.03$). These findings are consistent with causal roles for environmental polycyclic aromatic hydrocarbons and genetically encoded variants in enzymes whose actions lead to the production of activated polycyclic aromatic hydrocarbon metabolites.

Introduction

Colorectal cancer has been inconsistently associated with dietary and smoking-related exposure to PAHs[3] (1, 2). The carcinogenic potential of xenobiotic compounds such as PAHs may be determined by both the exposure dose and the extent of subsequent activation and inactivation by metabolic enzymes. Because substantial inter-individual variation has been demonstrated in both levels and activities of some relevant metabolic enzymes, PAH-mediated effects on the risk of colon cancer may be more readily detected in analyses that account for both the level of PAH exposure and individual variations in metabolic enzymes. Several recent studies address the possibility that functional polymorphisms in genes involved in PAH metabolism may predispose to the risk of colon cancer. For example, common loss-of-function deletion mutations have been described for genes encoding both GSTM1 and GSTT1, enzymes that catalyze the conjugation of glutathione to xenobiotics and thereby facilitate their inactivation and excretion. No clear pattern has emerged from nine published studies investigating possible associations between these deletion genotypes and carcinoma or adenoma of the colon (3), perhaps because most of the studies did not address PAH-gene or gene-gene interactions, or because protective effects are conferred by one or more unmeasured genes coexpressed with the measured genes or by unmeasured alleles in linkage disequilibrium with the measured genotypes. GSTM1 and four additional μ class GSTs (GSTM2-M5) constitute the known human μ class GSTs, which have overlapping substrate specificities. The genes encoding these GSTs are situated in tandem, so their alleles may be in linkage disequilibrium. The substrate specificity of human GSTM3 has not been characterized. However, Inskip *et al.* (4) found that the **B* allele of *GSTM3* is in linkage disequilibrium with *GSTM1*A*, an expressed allele of *GSTM1*, and Nakajima *et al.* (5) showed that the expression of GSTM3 was significantly correlated with GSTM1 in lung cytosol, where the corresponding GST proteins may be involved in the first-pass metabolism of PAHs. Harrison *et al.* (6) investigated the association between colon cancer and polymorphisms in the gene encoding mEH, another enzyme involved in PAH metabolism. mEH is a bifunctional protein that is able to mediate bile acid transport (7) and hydrolyze a broad range of epoxide substrates. Although bile acids have been implicated in the etiology of colon cancer, the current analysis does not formally address the bile acid transporter function. Instead, it is focused on epoxide hydrolase activity, through which mEH plays a central role in the metabolism of several PAH procarcinogens (8). mEH is expressed in most tissue and is highly expressed in liver, the primary site of detoxification reactions. Available data (9) suggest there is a 2- to 10-fold range in the amount of hepatic mEH among most of the population (although a few individuals have more extreme values), and total hepatic mEH activity is associated with level of mEH protein (10). Two *mEH* polymorphisms (population frequency, >1%) that encode amino acid substitutions have been described: the exon 3 residue 113 T to C mutation; and the exon 4 residue 139 A to G mutation. Although some reports refer to the resulting enzyme variants as "slow" and "fast," respectively, *in vitro* data show only minimal differences in specific activities and suggest that differences in total activity result largely from differing stabilities of the polymorphic proteins and other processes determining mEH protein levels (11, 12). We infer that alleles at these polymorphisms encode proteins with putatively high or low stability, and we interpret the genotypes they define as proxy measures of total mEH activity. In an analysis that did not address PAH exposure or GST genotypes, Harrison *et al.* (6) found that the frequency of the exon 3 putatively less stable (slow) *mEH* variant was more frequent among colon cancer patients than among blood donor controls (OR, 4.1; 95% CI, 1.9–9.2); they found no difference between cases and controls in the prevalence of the exon 4 putatively more stable (fast) *mEH* variant. We investigated the association between colorectal adenoma, a precursor to colon carcinoma, and total mEH activity as predicted by the exon 3 and 4 mEH polymorphisms as well as a novel intron 1 polymorphism. Our analyses account for possible interactions between predicted mEH activ-

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[3] The abbreviations used are: PAH, polycyclic aromatic hydrocarbon; GST, glutathione *S*-transferase; mEH, microsomal epoxide hydrolase; OR, odds ratio; CI, confidence interval.

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ity and both PAH exposure (as measured by tobacco smoking and consumption of well-done red meat) and a polymorphism in the gene encoding GSTM3.

Subjects and Methods

Study Population. The study population has already been described in detail (13). Eligible men and women underwent screening sigmoidoscopy at Kaiser Permanente's Bellflower or Sunset medical centers; were aged 50–74; were free of invasive cancer, inflammatory bowel disease, and familial polyposis; had no previous bowel surgery; were residents of Los Angeles or Orange County; and had no physical or mental disability precluding an interview. Cases were individuals diagnosed for the first time with one or more histologically confirmed adenoma. Fifteen cases who had carcinoma *in situ* in addition to adenomas were excluded. Controls had no polyps of any type at sigmoidoscopy, had no history of polyps, and were individually matched to cases by gender, age (within 5-year category), date of sigmoidoscopy (within 3-month category), and Kaiser center. Recruitment began after appropriate Institutional Review Board approvals were obtained. All subjects signed a written informed consent form approved by the Institutional Review Board, and all data have been anonymized. Participants provided data describing smoking history and diet in the year preceding sigmoidoscopy during an in-person interview. If the control initially matched to a case was not interviewed, a replacement control was identified. The response rate for interview data (number interviewed/number eligible) was 84% among cases, and 82% among controls. The response rate for blood draws was 84% for cases and 81% for controls. Race was nearly identically distributed among cases and controls, with 56% of cases and 54% of controls being Caucasian, 17% of cases and controls African-American, 17% of cases and 18% of controls Latino, and 10% of cases and 11% of controls Asian/Pacific Islander.

Laboratory Analysis. Genotyping reactions used 12 ng of genomic DNA isolated from blood lymphocytes. PCR products were loaded onto agarose gel, electrophoresed, stained with ethidium bromide, and visualized under a UV transilluminator.

In separate studies, a novel sequence variant in a *mEH* intron 1 region involved in transcriptional regulation was recently shown to be associated with a >85% reduction of mRNA and a >95% reduction in protein levels.[4] Genotypes for the *mEH* intron 1 variant were determined by PCR-amplification of specific alleles, which distinguished homozygous wild-type (11), heterozygous (12), and homozygous variant (22) alleles.

The *mEH* exon 3 T to C substitution at codon 113 leads to a transition substitution of tyrosine by histidine (Y113H) and elimination of the *Eco*RV restriction site (GATATC) present in the wild-type allele. The *mEH* exon 4 A to G substitution at codon 139 leads to a transition substitution of histidine by arginine (H139R) and creation of an *Rsa*I restriction site (GT/AC) not present in the wild-type sequence. We discriminated between alternate alleles by amplifying a PCR product that includes the polymorphic nucleotide, submitting the product to digestion with the appropriate restriction enzyme, then scoring alleles on the basis of fragment size as described by Harrison *et al.* (6).

The allele *GSTM3*A* (**A*), which is 3 bp longer than the alternate allele *GSTM3*B* (**B*), contains an Mnl I recognition site (CCTC(N)7/) not present in **B*. To distinguish between **A* and **B*, we amplified a 270–273-bp PCR product from the exon 6/7 region of *GSTM3*, subjected PCR products to digestion with Mnl I and electrophoresis, then scored alleles on the basis of fragment size (4, 14).

Statistical Analysis. We conducted χ^2 tests for Hardy-Weinberg equilibrium of alleles at the measured polymorphisms in the *mEH* and *GSTM3* genes. To test for linkage disequilibrium between pairs of *mEH* polymorphisms, we inferred haplotypes for all control subjects whose haplotypes were unambiguous (*i.e.,* for each pair analyzed, we excluded doubly heterozygous controls, 3–10% for each analysis). Ambiguous haplotypes were not assigned probabilistically, because we found Hardy-Weinberg disequilibrium among unambiguous haplotype pairs in several racial groups (χ^2; $P < 0.05$). We used standard logistic regression to estimate the race-adjusted OR for presence of the high-stability allele (exon 3 Y variant and exon 4 R variant) or high-transcript level allele (intron 1 wild-type) at one polymorphism given the allele in the other polymorphism of the haplotype. Race was coded using indicator variables for African-American, Latino, and Asian groups, with Caucasians as the referent group. We tested for heterogeneity in these "disequilibrium ORs" by including interaction variables between allele and race in the regression model. To estimate associations between measured polymorphisms and case-control status, we used unconditional logistic regression, controlling for the matching variables (age, gender, clinic, and exam date) and race. By using unconditional logistic regression we made use of all available genotypes in this dataset. Because matching yielded relatively large numbers of cases and controls within each stratum, an analysis using unconditional logistic regression adjusting for the matching variables leads to essentially the same results as a conditional analysis (15). Without postulating specific half-lives or kinetic parameters, we assumed that exonic alleles associated with more stable protein sequences and the intronic allele associated with a higher transcript level predict, in an additive fashion, higher protein levels and therefore higher total mEH activity. To study the combined effects of multiple *mEH* polymorphisms, we used these assumptions to define two measures of predicted (total) mEH activity. The two-locus measure was previously defined by Jourenkova- Mironova *et al.* (16), as follows. Defining the Y variant at exon 3 and the R variant at exon 4 as more stable, the "low" predicted level was assigned to individuals with 0 or 1, "medium" to individuals with 2, and "high" to individuals with 3 or 4 of the more stable alleles. (See Table 2, footnote *b*, for full enumeration.) For the three-locus measure, we assumed that the intron 1 wild-type allele associated with a higher transcript level also predicts a higher protein level, and using the above definitions for the exon 3 and 4 variants, we assigned "low" predicted activity to individuals with 0 to 3, "medium" to those with 4, and "high" to those with 5 or 6 alleles associated with higher stability or level of protein. (See Table 2, footnote *c*.) To test for possible modification of the effects of predicted mEH activity by smoking status (current smoker/never smoker), consumption of well-done red meat among never smokers (yes/no), and *GSTM3* genotype (**A*A*, **A*B*, or **B*B*), we included in our regression model the product of the variables for *mEH* and the exposure of interest, coding genotypes as linear trends (mEH: 0, low; 1, medium; 2, high; *GSTM3*: number of **A* alleles).

Results and Discussion

Genotype Frequencies. Genomic DNA samples from 88% of the interviewed cases (466) and 90% of the interviewed controls (509) were subjected to genotype analysis for the *mEH* intron 1, exon 3, and exon 4 polymorphisms, and the *GSTM3*A/*B* polymorphism. Resulting race-specific frequencies and counts of *mEH* genotypes among controls are shown in Table 1. We conducted tests for Hardy-Weinberg equilibrium for all four polymorphisms and found deviations only at the *mEH* exon 3 polymorphism. As shown in Table 1, these deviations were significant for Caucasians ($P = 0.0001$) and Asians ($P = 0.003$) and suggestive among African Americans ($P = 0.09$). The observed deviation may result from some degree of assortative mating in an admixed population, which is more apparent for the exon 3 polymorphism than for the others because the frequencies of exon 3 alleles differ more dramatically by race and are closer to 0.5. The departure does not appear to result from systematic laboratory error, inasmuch as the *Y* allele occurs more frequently than expected among Caucasians, whereas the *H* allele occurs more frequently than expected among Asian/Pacific Islanders. Results of pairwise tests for linkage disequilibrium among the three *mEH* polymorphisms suggest that all three loci may be in linkage disequilibrium with one another, although the pattern is not entirely clear. The test for linkage disequilibrium between exons 3 and 4, conducted among 904 of 1004 possible haplotypes (100 doubly heterozygous haplotypes, or 10%, were excluded), revealed that the exon 3 Y variant may be positively associated with the exon 4 *R* variant, as the race-adjusted OR for carrying the exon 4 *R* allele for a carrier of the exon 3 *Y* allele was 2.1 (CI, 1.3–3.3; unadjusted OR, 2.5). More striking linkage disequilibrium was detected between intron 1 and both exons 3 and 4. Among 974 haplotypes (30, or 3%, excluded), the intron 1 wild-type allele was negatively associated with the exon 3 Y variant (race adjusted OR, 0.55; CI, 0.27–0.80; unadjusted OR, 0.55). Among 960 haplo-

[4] Q-S. Zhu, W. Xing, B. Qian, P. von Dippe, B. L. Shneider, V. L. Fox, D. Levy. Mutations in intron1 and an upstream HNF-3 site in human EPHX1 encoding microsomal epoxide hydrolase are associated with extreme reduction in gene expression and with defective hepatic bile acid uptake, manuscript in preparation.

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Table 1 *mEH genotype frequencies (by percentage) by race for controls*

	Caucasian (n = 277)		African-American (n = 84)		Latino (n = 93)		Asian/Pacific Islander (n = 55)	
	Observed	(%)	Observed	(%)	Observed	(%)	Observed	(%)
mEH								
Introl 1								
11	218	(79.6)	81	(96.4)	75	(80.6)	43	(78.2)
12	52	(19.0)	3	(3.6)	16	(17.2)	10	(18.2)
22	4	(1.5)	0	(0.0)	2	(2.2)	2	(3.6)
Missing	3							
$P_{HWE}{}^{a}$		NS		NS		NS		NS
mEH								
Exon 3								
HH	46	(16.8)	9	(10.7)	23	(22.8)	27	(50.9)
HY	90	(33.0)	24	(28.6)	48	(52.2)	13	(24.5)
YY	137	(50.2)	51	(60.7)	21	(25.0)	13	(24.5)
Missing	4				1		2	
$P_{HWE}{}^{a}$		0.0001		0.09		NS		0.003
mEH								
Exon 4								
HH	180	(66.2)	49	(59.0)	70	(76.1)	44	(81.5)
HR	80	(29.4)	29	(34.9)	22	(23.9)	10	(18.5)
RR	12	(4.4)	5	(6.0)	0	(0.0)	0	(0.0)
Missing	5		1		1		1	
$P_{HWE}{}^{a}$		NS		NS		NS		NS
GTSM3								
**A*A*	6	(2.2)	31	(37.3)	3	(3.3)	0	(0.0)
**A*B*	60	(22.4)	37	(44.6)	20	(21.7)	1	(1.9)
**B*B*	202	(75.4)	15	(18.1)	69	(75.0)	51	(98.1)
Missing	9		1		1		3	
$P_{HWE}{}^{a}$		NS		NS		NS		NS

[a] P calculated in χ^2 test for Hardy-Weinberg equilibrium. NS, not significant ($P > 0.2$).

types (42, or 4%, excluded), the intron 1 wild-type allele was positively associated with the exon 4 R variant (race-adjusted OR, 13.9; CI, 2.2–80.0; unadjusted OR, 15.7). To better understand these linkage disequilibrium relationships, they should be reexamined in larger numbers of subjects.

***mEH*-Adenoma Associations.** We examined associations between *mEH* variants and the occurrence of adenoma in several ways. To compare our data with those reported by Harrison *et al.* (6), first we estimated ORs for each separate genotype. Then, we estimated associations between predicted mEH activity and adenoma using each of the two composite measures. The two-locus measure can be used to compare these findings with previous reports, whereas the three-locus measure more fully describes these data. Resulting ORs appear in Table 2.

These results suggest either no overall association, or a slightly elevated frequency among adenoma cases of *mEH* variants predicting higher activity. Both interpretations are inconsistent with the finding of Harrison *et al.* (6) that the lower stability exon 3 H variant was more prevalent among colon cancer cases, although they reported no association with the exon 4 variant. The validity of the study reported by Harrison *et al.* is difficult to judge, because of questions about the comparability of blood donor controls to colon cancer cases, where blood donors may not reflect the source population of cases if there are different referral patterns for cases than for subjects providing blood donations. In addition, controls were 18–65 years of age and may have included individuals with a history of colon cancer. If valid, a possible explanation for the inconsistency is that although colorectal adenoma is the outcome of the present study, carcinoma was the outcome addressed by Harrison *et al.*, and the exon 3 H variant is involved in progression from adenoma to carcinoma of the colon but not in occurrence of adenoma itself. However, this interpretation is contradicted by the direction of the *mEH* effect in the interactions we describe below. Another possible explanation is that the findings in Table 1 and the associations reported by Harrison *et al.* combine estimates over levels of important effect modifiers that are weighted differently in the two study populations. To incorporate into the analysis variables that might be important effect modifiers, we attempted to identify genetic and environmental factors that might work with mEH to produce adenomas by considering the biological function of mEH.

Modifiers of *mEH*-Adenoma Associations. We assumed that any increased risk of adenoma caused by PAH exposure is largely determined by concentrations of activated PAH metabolites, and that these concentrations are, in turn, determined by two general factors: (*a*) the amount of PAH taken into the body; and (*b*) the relative concentrations and activities of enzymes that catalyze the synthesis and breakdown of activated metabolites, conditions determined in part by specific forms of genes encoding these enzymes. To address the possibility that the hydrolase activity of mEH is a determinant of risk of adenoma, we considered metabolic pathways in which mEH hydrolyzes metabolites of PAHs. We focused on a simplified pathway in which benzo(*a*)pyrene is converted to several of its metabolites, illustrated in Fig. 1.

As shown in Fig. 1, this set of reactions can create two different activated metabolites, benzo(*a*)pyrene 7,8-epoxide (epoxide) and benzo(*a*)pyrene 7,8 dihydrodiol 9,10-epoxide (diol-epoxide), an isomer of which has been shown experimentally to exhibit significantly greater mutagenicity. The epoxide can be converted to benzo(*a*)pyrene 7,8 dihydrodiol (diol) by mEH-catalyzed hydrolysis, and the diol can subsequently be converted to the diol-epoxide. Therefore, although higher mEH activity may more readily deplete the epoxide fraction, it may also facilitate enrichment of the diol-epoxide fraction of benzo(*a*)pyrene metabolites. On the basis of this pathway, we reasoned that any of the following factors could modify the effect of mEH on the risk of adenomas by modifying levels of either the epoxide or the diol-epoxide: (*a*) the level of PAH exposure; (*b*) the concentration and activity of those cytochrome P450 enzymes that direct the synthesis of either the epoxide or the diol-epoxide; and (*c*) the concentration and activity of those GSTs that direct glutathione conjugation of either the epoxide or the diol-epoxide. Current knowledge does not allow us to identify with certainty all of the cytochrome P450s and GSTs that may participate in this pathway or to identify their important functional polymorphisms. Given these constraints and the sample size limits of this dataset to address multifactorial problems, we chose to analyze as

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Table 2 *Distribution of mEH polymorphisms and predicted mEH activity in adenoma cases and controls*

Genotype	No. of cases (%)	No. of controls (%)	OR[a] (95% CI)
mEH intron 1			
11	376 (81.0)	417 (82.4)	1.0 (reference)
12	80 (17.2)	81 (16.0)	1.08 (0.76–1.53)
22	8 (1.7)	8 (1.6)	1.11 (0.40–3.09)
Total	464	506	
Two-sided trend *P*	0.64		
mEH exon 3			
HH	93 (20.2)	105 (20.9)	1.0 (reference)
HY	136 (29.5)	175 (34.9)	0.89 (0.62–1.30)
YY	232 (50.3)	222 (44.2)	1.21 (0.85–1.73)
Total	461	502	
Two-sided trend *P*	0.14		
mEH exon 4			
HH	302 (65.5)	343 (68.5)	1.0 (reference)
HR	136 (29.5)	141 (28.1)	1.09 (0.82–1.46)
RR	23 (5.0)	17 (3.4)	1.54 (0.80–3.01)
Total	461	501	
Two-sided trend *P*	0.22		
Predicted activity,[b] Two-locus measure Exon 3/exon 4)			
Low	189 (41.1)	227 (45.6)	1.0 (reference)
Medium	175 (38.0)	182 (36.5)	1.17 (0.87–1.57)
High	96 (20.9)	89 (17.9)	1.33 (0.93–1.91)
Total	460	498	
Two-sided trend *P*	0.11		
Predicted activity,[c] Three-locus measure (Intron/exon 3/exon 4)			
Low	225 (48.9)	272 (54.6)	1.0 (reference)
Medium	153 (33.3)	150 (30.1)	1.28 (0.95–1.72)
High	82 (17.8)	76 (15.3)	1.36 (0.93–1.98)
Total	460	498	
Two-sided trend *P*	0.06		

[a] Adjusted for age, gender, race, date of sigmoidoscopy, and Kaiser center.
[b] Low, 0 or 1 more stable alleles (*Y* at exon 3 or *R* at exon 4), combined: *HH/HH*, *HH/HR*, or *HY/HH*. Medium, 2 more stable alleles: *HH/RR, HY/HR,* or *YY/HH*. High, 3 or 4 more stable alleles: *HY/RR*, *YY/HR*, or *YY/RR*.
[c] Low, 0–3 more stable alleles (*Y* or *R*) at exon 3 and exon 4 or high-transcript alleles (1) at intron 1, combined. Medium, 4 more stable or high-transcript alleles. High, 5 or 6 more stable or high-transcript alleles.

potential effect modifiers the best proxy measures of PAH exposure available in these data (subjects' histories of cigarette smoking and well-done red meat consumption) and a polymorphism in a GST-encoding gene, *GSTM3*A/*B*. A previous report (16) suggested that this *GSTM3* polymorphism may be an important modifier of associations between *mEH* polymorphisms and smoking-related disease. However, the functional significance of the *GSTM3* polymorphism is not clear, and reported effects may arise from linkage disequilibrium with unmeasured variants in the *GSTM3* region, which contains five *GST* μ class genes.

The results of these analyses, presented in Table 3, strongly suggest that each of these factors (smoking, consumption of well-done red meat, and *GSTM3*) may modify the effect of *mEH* on the occurrence of adenoma, suggesting that mEH activity may be a much more important determinant of risk of adenoma in some subgroups. We first examined the *mEH*-adenoma association in never *versus* current smokers, eliminating past smokers from the analysis in an attempt to define groups with the largest possible differences in PAH exposure. Modification of the *mEH* effect by smoking is illustrated by effect estimates for groups defined by smoking and *mEH*. Defining the reference group as never smokers with low predicted mEH activity (Table 3A) we estimated the OR among current smokers with high predicted mEH stability to be 4.27 (CI, 1.68–10.81). We observed a similar OR, 4.06 (CI, 1.53–10.77) when we repeated this analysis using mEH activity predicted by all three loci (full results not shown). To explore additional modification of *mEH* effects by PAH exposure, as measured by consumption of well-done red meat, we conducted an analysis among never smokers who eat meat. We anticipated that a *mEH*-well done red meat interaction may be most readily observed among this subgroup because of the minimal presence among them of any uncontrolled effects of PAH intake from tobacco smoke (which is presumed to be a stronger source of PAHs) or deliberate dietary practices such as vegetarianism. In an analysis of *mEH*, using for the reference group individuals with low predicted mEH activity who do not regularly eat their meat well-done (full results in Table 3B), the OR was 2.47 (CI, 0.99–6.19) for those who do regularly eat their meat well-done and have high predicted mEH activity. The OR for this group was 4.71 (CI, 1.65–13.42) in an analysis using the three-locus measure of predicted mEH activity (full results not shown). Finally, we examined the *mEH* association among groups with genotypes defined by the *GSTM3*A/*B* polymorphism (Table 3C). With subjects with low predicted mEH activity and the **B*B* genotype as the reference group, we estimated an OR of 2.60 (CI, 1.28–5.28) for individuals with high mEH activity and the **A*A* genotype. The OR for the same group was 4.27 (CI, 1.68–10.81) in an analysis using the three-locus measure of predicted mEH activity (full results not shown). In addition to the analyses presented in Table 3, one may also calculate ORs for specific exposures within strata of mEH activity. For example, among individuals with low predicted mEH activity, the OR for current smokers *versus* never smokers was 1.58, whereas among individuals with medium predicted activity this OR was 2.78, and among those with high predicted activity, it was 3.78. Similarly, among individuals with low predicted mEH activity, the OR for those who usually ate well-done red meat *versus* those who usually ate red meat not well-done was 1.01, whereas among those with medium predicted mEH activity, this OR was 1.49, and among those with high predicted mEH activity, this OR was 4.33. These findings are consistent with the hypothesis that smoking and meat consumption are causes of colon cancer and convey greater risks to individuals with higher mEH activity. If this is true, then inconsistencies in previous reports of associations between smoking or meat consumption and colon cancer may have occurred, at least in part, because earlier reports did not account

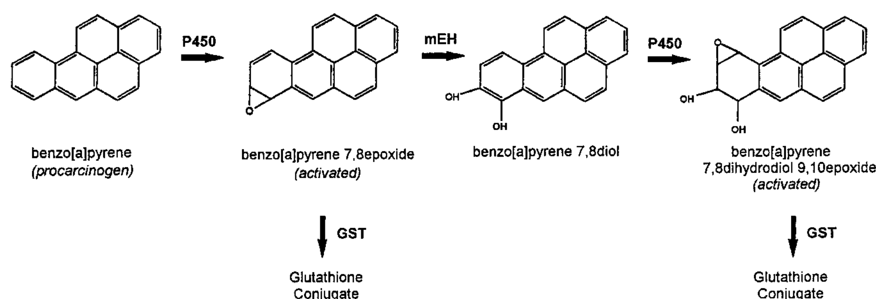

Fig. 1. Simplified pathway illustrating enzymatic conversion of a PAH procarcinogen [benzo(*a*)pyrene] to activated metabolites [benzo(*a*)pyrene 7,8 epoxide and benzo(*a*) 7,8 dihydrodiol 9,10 epoxide]. *mEH*, activity; *P450*, activity of unspecified cytochrome P450; *GST*, activity of unspecified GST.

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Table 3 *Distribution of predicted mEH activity by levels of three effect modifiers mEH activity[a] predicted by exon 3 and exon 4 genotypes*

	Low	Medium	High
A. Smoking status (Excluding past smokers)			
Never smoker			
Case/Control	80/97	53/74	32/41
OR[b] (95% CI)	1.00 (reference)	0.92 (0.57–1.49)	1.13 (0.63–2.03)
Current smoker			
Case/Control	27/24	44/25	19/8
OR[b] (95% CI)	1.58 (0.82–3.05)	2.56 (1.38–4.74)	4.27 (1.68–10.81)
			Interaction, $P = 0.11$
B. Usual preparation of cooked meat, among never smokers who eat meat			
Not well-done			
Case/Control	42/48	27/42	11/24
OR[b] (95% CI)	1.00 (reference)	0.81 (0.41–1.62)	0.57 (0.24–1.35)
Well-done			
Case/Control	36/43	24/26	19/13
OR[b] (95% CI)	1.01 (0.53–1.93)	1.21 (0.58–2.53)	2.47 (0.99–6.19)
			Interaction, $P = 0.03$
C. *GSTM3*A/*B* Genotype among all participants			
**B*B*			
Case/Control	142/163	114/116	45/55
OR[b] (95% CI)	1.00 (reference)	1.02 (0.82–1.26)	1.03 (0.67–1.58)
**A*B*			
Case/Control	37/46	43/49	35/23
OR[b] (95% CI)	0.86 (0.62–1.21)	1.19 (0.87–1.62)	1.64 (1.06–2.52)
**A*A*			
Case/Control	9/15	18/16	16/9
OR[b] (95% CI)	0.75 (0.38–1.47)	1.64 (1.06–2.52)	2.60 (1.28–5.28)
			Interaction, $P = 0.03$

[a] Low, 0 or 1 more stable alleles (*Y* at exon 3 or *R* at exon 4), combined: *HH/HH, HH/HR,* or *HY/HH*. Medium, 2 more stable alleles: *HH/RR, HY/HR,* or *YY/HH*. High, 3 or 4 more stable alleles: *HY/RR, YY/HR,* or *YY/RR.*

[b] Adjusted for age, gender, race, date of sigmoidoscopy, and Kaiser center.

for the effects of mEH activity or additional important metabolic enzymes. Given our results suggesting possible gene-environment and gene-gene interactions, a logical extension is to next study gene-gene-environment interactions predicted by our data (*e.g., mEH-GSTM3*-smoking/red meat interactions); however, our current sample size precludes an informative analysis of three-way interactions.

In summary, our results suggest that mEH stability may, in fact, be associated with a risk of colorectal adenomas, but that one must account for environmental measures of exposure to PAHs, such as smoking and consumption of well-done red meat, and other modifying genes, such as *GSTM3*, before the effects of *mEH* become apparent. The fact that the *mEH* effect is modified by smoking, well-done red meat, and *GSTM3* genotypes suggests that at least part of the increased risk of colorectal adenoma associated with smoking and consumption of well-done red meat is attributable to PAHs. The biochemical pathways in which mEH participates involve numerous enzymes, only some of which we studied here. The role of mEH itself may be quite complex, as the enzyme has broad substrate specificity and tissue distribution (17), its transcript can exist as multiple splice variants (18), there are additional known sequence variants not measured in this study (19), and there may be substantial linkage disequilibrium within the gene. In addition, expression of both mEH and other enzymes in these pathways have been shown to be both induced and inhibited by exogenous compounds, and *mEH* is subject to substrate-specific activation and inactivation by numerous compounds (17). We believe we can better elucidate the effects of candidate genes by considering them in the context of a given metabolic pathway and considering other factors, both genetic and environmental, in that pathway. The work presented here is an example of the initial application of this pathway-guided approach to the epidemiological study of *mEH*.

Larger epidemiological studies are needed to confirm the results we observed and to assess the role of genes that encode additional enzymes participating in the same mEH-mediated metabolic pathways and additional forms of environmental procarcinogens on which these enzymes may act. These studies will be informed further by basic research identifying tissue compartments in which relevant reactions occur and the tissue distribution of activated metabolites.

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A Case-Control Study of Microsomal Epoxide Hydrolase, Smoking, Meat Consumption, Glutathione *S*-Transferase M3, and Risk of Colorectal Adenomas

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