

Null Results in Brief

Microsomal Epoxide Hydrolase Polymorphisms Are Not Associated with Colon Cancer Risk

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Introduction

Epidemiologic studies have implicated exposure to tobacco smoke and high intakes of cooked, broiled, or well-done meats in the increased risk of colorectal cancers (1-4). Microsomal epoxide hydrolase (mEH) is a phase II biotransformation enzyme which detoxifies epoxides, including carcinogens such as polycyclic aromatic hydrocarbons found in cigarette smoke and cooked meats (5). A tyrosine to histidine substitution in exon 3 (*Y113H*) of the *mEH* gene decreases *in vitro* enzyme activity by 40%, whereas a histidine to arginine substitution in exon 4 (*H139R*) increases *in vitro* enzyme activity by 25% (6). Smaller epidemiologic studies evaluating the relationship between the *mEH* polymorphisms and risk of colorectal cancer and its precursors have been inconclusive (7-9). We evaluated the risk of colon cancer associated with both the *mEH* *Y113H* and *H139R* genotypes in a large case-control study.

Materials and Methods

Methods for selection of cases and controls and data collection have been described in detail elsewhere (10-12). Briefly, participants were subjects from the Kaiser Permanente Medical Care Program of Northern California, an eight-county area in Utah, and the metropolitan Twin Cities area of Minnesota. Eligibility criteria have been previously described (10). Controls who had never had a previous colorectal tumor were selected from Kaiser Permanente Medical Care Program membership lists in California; driver's license lists, random-digit-dialing, or Centers for Medicare and Medicaid Services lists for Utah; and driver's license or state identification lists in Minnesota.

***mEH* Genotyping.** The *mEH* *Y113H*, *H139R* polymorphisms were detected using the 5' nuclease assay on a 7900HT sequence detection system (Applied Biosystems, Foster City, CA). Primers and probes and PCR core reagents were purchased from Applied Biosystems. The assays were validated by genotyping 100 individuals by both 5' nuclease assay and RFLP or sequencing. There were no discrepancies between the two assays. Genotyping was done in 20 μ L reactions

containing 1 \times Taqman PCR core reagents 4 mmol/L MgCl₂, 200 nmol/L primers (*Y113H*: 5'CTGGAAGAAGCAGGTG-AGCAGGTGGAGATT3', 5'TGGCTGGCGTTTGGCAA3'; *H139R*: 5'TCCACCCCTGACTGTGCTCTGT3', 5'TGGGATGATGGGATGATCTTATAAACTCGTAGAAA3'), 100 nmol/L probes (*Y113*: VIC-5'TCAACAGATACCCTCACT3'-NFQ; *Y113H*: 6FAM-5'AACAGACACCCTCACT3'-NFQ; *H139*: VIC-5'CAGGCCATACCCCGA3'-NFQ; *Y139R*: FAM-5'AGGCC-AGGCCGTACCCCGA3'-NFQ), and 3 ng DNA. Amplification cycles were 50°C for 5 minutes, 95°C for 10 minutes, and 40 cycles of 95°C for 15 seconds and 60°C for 60 seconds. Positive controls for all the genotypes and reagent controls were included in each plate. Genotyping of 94 randomly selected samples was repeated for each polymorphism. There were no discrepancies.

Statistical Methods. Unconditional logistic regression models were used. Multivariate adjustment included age, sex, body mass index (kg/m²), vigorous physical activity index (13), regular use of aspirin or nonsteroidal anti-inflammatory drugs, and usual number of cigarettes smoked per day. This study had 90% power to detect an odds ratio (OR) of 1.4 for the main effect comparing homozygous wild-type *mEH* *Y113YY* with homozygous variant *mEH* *Y113HH* genotype, and 80% power to detect an OR of 1.4 for the equivalent comparison within the *H139R* genotype.

Results

Of 4,403 eligible participants with valid data for the study, 3,553 participants with available DNA (1,593 cases and 1,960 controls) were genotyped for both the *mEH* *Y113H* and *H139R* polymorphisms. Characteristics of the study population have been described elsewhere (14). Genotype frequencies for both the *Y113H* and *H139R* polymorphic sites were in Hardy-Weinberg equilibrium among the controls and among the cases, and did not vary significantly between cases and controls. Frequencies for both the *Y113H* *H* allele (0.27 for cases; 0.32 for controls) and *H139R* *K* allele (0.17 for cases; 0.22 for controls) are consistent with previously reported genotype frequencies (7, 8, 15-19).

Risks of colon cancer associated with the *Y113H* and *H139R* genotypes, combined genotypes, and imputed phenotypes as proposed by Smith and Harrison (19) are summarized in Table 1. Greater usual number of cigarettes smoked increased risk of colon cancer overall, especially when restricted to microsatellite instability-positive colon cancers; however, tests for trend did not identify significant differences by *mEH* *Y113H* or *H139R* genotypes. The risk estimates did not vary significantly when stratified by total

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Table 1. Risk of colon cancer associated with *mEH* genotypes

<i>mEH</i> genotype	Main effects*	Usual number of cigarettes smoked per day*			Total mutagen index* [†]		
		None	≤20	>20	0-451	452-831	≥832
<i>Y113H</i>							
<i>Y/Y</i>	1.0 (ref.) 789/991	1.0 (ref.) 330/473	1.2 (0.96-1.5) 305/380	1.6 (1.2-2.1) 154/138	1.0 (ref.) 223/315	1.2 (0.9-1.5) 284/334	1.1 (0.9-1.4) 282/342
<i>Y/H</i>	1.0 (0.9-1.2) 655/795	1.0 (0.8-1.3) 272/376	1.3 (1.0-1.6) 269/314	1.5 (1.1-2.1) 114/105	1.0 (0.8-1.3) 193/265	1.2 (0.9-1.6) 223/260	1.2 (0.9-1.5) 239/270
<i>H/H</i>	1.1 (0.8-1.4) 149/174	1.1 (0.7-1.6) 55/74	1.3 (0.90-1.9) 66/75	1.5 (0.9-2.7) 28/25	1.2 (0.8-1.8) 48/58	1.0 (0.6-1.5) 44/63	1.4 (0.9-2.1) 57/53
<i>H139R</i>							
<i>H/H</i>	1.0 (ref.) 1,051/1,267	1.0 (ref.) 436/610	1.3 (1.1-1.5) 425/481	1.5 (1.2-1.9) 190/176	1.0 (ref.) 304/415	1.2 (0.9-1.4) 371/433	1.2 (0.9-1.4) 376/419
<i>H/R</i>	1.0 (0.8-1.1) 479/605	1.0 (0.8-1.2) 194/276	1.1 (0.9-1.4) 190/248	1.6 (1.2-2.3) 95/81	1.0 (0.8-1.3) 146/192	1.1 (0.8-1.4) 154/191	1.0 (0.8-1.4) 179/222
<i>R/R</i>	0.8 (0.6-1.2) 63/88	1.0 (0.6-1.7) 27/37	0.9 (0.5-1.5) 25/40	1.4 (0.6-3.2) 11/11	0.6 (0.3-1.2) 4/31	1.0 (0.6-1.8) 26/33	1.2 (0.7-2.2) 23/24
Imputed phenotypes[‡]							
Rapid	1.0 (0.8-1.2) 282/351	1.0 (0.8-1.4) 109/151	1.1 (0.8-1.5) 113/148	1.7 (1.1-2.6) 60/52	1.1 (0.7-1.5) 81/107	1.3 (0.9-1.7) 103/116	1.1 (0.8-1.5) 98/128
Normal	1.0 (ref.) 694/882	1.0 (ref.) 306/441	1.2 (0.9-1.5) 261/329	1.6 (1.2-2.2) 127/112	1.0 (ref.) 197/288	1.2 (0.9-1.5) 236/290	1.2 (0.9-1.5) 261/304
Slow	1.1 (0.9-1.2) 468/553	1.0 (0.8-1.3) 187/257	1.4 (1.1-1.7) 200/217	1.4 (1.0-2.0) 81/79	1.1 (0.8-1.4) 138/185	1.3 (0.97-1.7) 168/188	1.2 (0.9-1.6) 162/180
Very slow	1.1 (0.8-1.4) 149/174	1.1 (0.7-1.6) 55/74	1.3 (0.9-1.9) 66/75	1.5 (0.9-2.7) 28/25	1.2 (0.8-1.8) 48/58	1.0 (0.7-1.6) 44/63	1.4 (0.9-2.2) 57/53
Combined genotypes							
<i>113YY/139HH</i>	1.0 (ref.) 507/640						
<i>113YY/139HR</i>	1.0 (0.8-1.2) 247/310						
<i>113YH/139HH</i>	1.1 (0.9-1.3) 444/513						
<i>113YH/139HR</i>	1.0 (0.8-1.2) 187/242						
<i>113HH/139HH or 139HR</i>	1.1 (0.8-1.4) 145/167						
<i>Any Y113H/139RR</i>	0.9 (0.6-1.2) 63/88						

NOTE: All estimates are multivariate adjusted for age, sex, body mass index (kg/m²), vigorous physical activity index, regular use of aspirin or nonsteroidal anti-inflammatory drugs, and usual number of cigarettes smoked per day where appropriate.

*Reported as OR (95% confidence interval), number of cases/number of controls.

[†]Total mutagen index is calculated as the frequency of cooked red meat, poultry, and fish consumption plus the use of drippings multiplied by usual doneness of meat (1, rare; 2, medium-rare; 3, medium-well; 4, well done) and the microwave factor (1, never used; 0.75, sometimes used; 0.5, often used; 0.25, always used; ref. 20).

[‡]Imputed phenotype, as classified by Smith and Harrison (19): rapid, *113YY/139HR* or *113YY/139RR*; normal, *113YY/139HH* or *113YH/139HR*; slow, *113YH/139HH* or *113YH/139RR*; and very slow, *113HH/139HH*.

mutagen index (20), dietary intake of red or white meat, tumor site (proximal versus distal), or genotype for the *NAT2*, *GSTM1*, or *CYP1A1* biotransformation enzymes (data not shown).

Discussion

Previous reports evaluating the association between *mEH* *Y113H* genotype and colorectal cancer have been contradictory. Harrison et al. (7) reported that the *113HH* genotype was associated with an increased risk of colon cancer (OR, 3.8; 95% confidence interval, 1.8-8.0), whereas Sachse et al. (9) found the *113HH* genotype to be associated with a decreased risk of colorectal cancer (OR, 0.7; 95% confidence interval, 0.5-0.95). Controls for the studies by Sachse et al. (9) and Harrison et al. (7) were not in Hardy-Weinberg equilibrium for the *Y113H* polymorphism, as has been frequently reported in studies using PCR-RFLP *mEH* *Y113H* genotyping methods (6, 9, 16). Neither group evaluated whether environmental factors such as smoking or well-cooked meat intake modified their findings.

With regard to the *H139R* polymorphism, analyses by Harrison et al. (7), Sachse et al. (9), and Mitrou et al. (8) all failed to find an association between genotype and risk of

colorectal cancer. However, among smokers, Mitrou et al. reported an increased risk of colorectal and distal colon cancers with the *139RR* genotype (OR, 2.9; 95% confidence interval, 1.3-6.0 and OR, 3.7; 95% confidence interval, 1.6-8.2, respectively).

Both Sachse et al. (9) and Harrison et al. (7) reported a significant difference in *Y113H* genotype frequencies between cases and controls, suggesting that *Y113H* genotype may be a susceptibility factor in the development of colorectal cancer. These findings of an altered risk were not confirmed in our study, and may be partially attributable to the fact that the previous studies included cases of rectal cancers, whereas rectal cancer was an exclusion criterion for our study.

It would be expected that *mEH* genotypes would alter risk of colon cancer under specific conditions such as exposure to cigarette smoke and dietary mutagens. However, most of the previous studies did not explore these interactions. This study, which found no association between *mEH* *Y113H* or *H139R* genotype and colon cancer risk, is the largest to date, and included evaluation of exposure to cigarette smoke and well-cooked meats. Although we did not observe any difference by *mEH* genotype, environmental exposure to cigarette smoke and dietary mutagens have predicted risk of colon cancer and adenomatous and hyperplastic polyps in our past research (15, 20-22).

Studies of adenomatous polyps, possible precursors of colorectal cancer, have similarly failed to find an association between *mEH* genotype and risk of polyps. However, some have observed interactions with smoking and cooked meat by *mEH* genotype (15, 16, 23, 24).

We conclude that the *mEH Y113H* and *H139R* genotypes do not affect risk of colon cancer, and may only be a factor in the development of adenomatous polyps (earlier stages of colon carcinogenesis) under conditions of elevated carcinogen exposure.

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References

- Potter JD. Colorectal cancer: molecules and populations. *J Natl Cancer Inst* 1999;91:916–32.
- Sinha R, Rothman N. Role of well-done, grilled red meat, heterocyclic amines (HCAs) in the etiology of human cancer. *Cancer Lett* 1999;143:189–94.
- Sinha R, Chow WH, Kulldorff M, et al. Well-done, grilled red meat increases the risk of colorectal adenomas. *Cancer Res* 1999;59:4320–4.
- Cross AJ, Sinha R. Meat-related mutagens/carcinogens in the etiology of colorectal cancer. *Environ Mol Mutagen* 2004;44:44–55.
- Seidegard J, DePierre JW. Microsomal epoxide hydrolase. Properties, regulation and function. *Biochim Biophys Acta* 1983;695:251–70.
- 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.
- Harrison DJ, Hubbard AL, MacMillan J, Wyllie AH, Smith CA. Microsomal epoxide hydrolase gene polymorphism and susceptibility to colon cancer. *Br J Cancer* 1999;79:168–71.
- Mitrou P, Watson M, Bingham S, Stebbings WS, Speakman CT, Loktionov A. NQO1 and mEH exon 4 (mEH4) gene polymorphisms, smoking and colorectal cancer risk. *IARC Sci Publ* 2002;156:495–7.
- Sachse C, Smith G, Wilkie MJ, et al. A pharmacogenetic study to investigate the role of dietary carcinogens in the etiology of colorectal cancer. *Carcinogenesis* 2002;23:1839–49.
- Slattery ML, Potter J, Caan B, et al. Energy balance and colon cancer—beyond physical activity. *Cancer Res* 1997;57:75–80.
- Slattery ML, Caan BJ, Duncan D, Berry TD, Coates A, Kerber R. A computerized diet history questionnaire for epidemiologic studies. *J Am Diet Assoc* 1994;94:761–6.
- Edwards S, Slattery ML, Mori M, et al. Objective system for interviewer performance evaluation for use in epidemiologic studies. *Am J Epidemiol* 1994;140:1020–8.
- Slattery ML, Edwards SL, Ma KN, Friedman GD, Potter JD. Physical activity and colon cancer: a public health perspective. *Ann Epidemiol* 1997;7:137–45.
- Curtin K, Bigler J, Slattery ML, Caan B, Potter JD, Ulrich CM. MTHFR C677T and A1298C polymorphisms: diet, estrogen, and risk of colon cancer. *Cancer Epidemiol Biomarkers Prev* 2004;13:285–92.
- 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.
- Cortessis V, Siegmund K, Chen Q, et al. A case-control study of microsomal epoxide hydrolase, smoking, meat consumption, glutathione S-transferase M3, and risk of colorectal adenomas. *Cancer Res* 2001;61:2381–5.
- Jourenkova-Mironova N, Mitrunen K, Bouchardy C, Dayer P, Benhamou S, Hirvonen A. High-activity microsomal epoxide hydrolase genotypes and the risk of oral, pharynx, and larynx cancers. *Cancer Res* 2000;60:534–6.
- Tiemersma EW, Omer RE, Bunschoten A, et al. Role of genetic polymorphism of glutathione-S-transferase T1 and microsomal epoxide hydrolase in aflatoxin-associated hepatocellular carcinoma. *Cancer Epidemiol Biomarkers Prev* 2001;10:785–91.
- Smith CA, Harrison DJ. Association between polymorphism in gene for microsomal epoxide hydrolase and susceptibility to emphysema. *Lancet* 1997;350:630–3.
- Kampman E, Slattery ML, Bigler J, et al. Meat consumption, genetic susceptibility, and colon cancer risk: a United States multicenter case-control study. *Cancer Epidemiol Biomarkers Prev* 1999;8:15–24.
- Slattery ML, Potter JD, Samowitz W, Bigler J, Caan B, Leppert M. NAT2, GSTM-1, cigarette smoking, and risk of colon cancer. *Cancer Epidemiol Biomarkers Prev* 1998;7:1079–84.
- Potter JD, Bigler J, Fosdick L, et al. Colorectal adenomatous and hyperplastic polyps: smoking and *N*-acetyltransferase 2 polymorphisms. *Cancer Epidemiol Biomarkers Prev* 1999;8:69–75.
- Tiemersma EW, Kloosterman J, Bunschoten A, Kok FJ, Kampman E. Role of EPHX genotype in the associations of smoking and diet with colorectal adenomas. *IARC Sci Publ* 2002;156:491–3.
- Tranah GJ, Giovannucci E, Ma J, Fuchs C, Hankinson SE, Hunter DJ. Epoxide hydrolase polymorphisms, cigarette smoking and risk of colorectal adenoma in the Nurses' Health Study and the Health Professionals Follow-up Study. *Carcinogenesis* 2004;25:1211–8.

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