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 μL reactions containing 1× Taqman PCR core reagents 4 mmol/L $MgCl_2$, 200 nmol/L primers (*Y113H*: 5′CTGGAAGAAGCAGGTGAGCAGGTGGAGATT3′, 5′TGGCTGGCGTTTTGCAA3′; *H139R*: 5′TCCACCCTGACTGTGCTCTGT3′, 5′TGGGATGATGGGATGATCTTATAAAACTCGTAGAAA3′), 100 nmol/L probes (*Y113*: VIC-5′TCAACAGATACCCTCACT3′-NFQ; *113H*: 6FAM-5′AACAGACACCCTCACT3′-NFQ; *H139*: VIC-5′CAGGCCATACCCCGA3′_NFQ; *139R*: FAM-5′AGGCCAGGCCGTACCCCGA3′-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/m^2), 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 113YY* with homozygous variant *mEH 113HH* 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 *H139K* polymorphisms. Characteristics of the study population have been described elsewhere (14). Genotype frequencies for both the *Y113H* and *H139K* 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 *H139K 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 *H139K* 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

mEH genotype	Main effects*	Usual number of cigarettes smoked per day*			Total mutagen index*,†		
		None	≤20	>20	0-451	452-831	≥832
Y113H							
Y/Y	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
Y/H	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
H/H	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
H139R							
H/H	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
H/R	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
R/R	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							
113YY/139HH	1.0 (ref.) 507/640						
113YY/139HR	1.0 (0.8-1.2) 247/310						
113YH/139HH	1.1 (0.9-1.3) 444/513						
113YH/139HR	1.0 (0.8-1.2) 187/242						
113HH/139HH or 139HR	1.1 (0.8-1.4) 145/167						
Any Y113H/139RR	0.9 (0.6-1.2) 63/88						

NOTE: All estimates are multivariate adjusted for age, sex, body mass index (kg/m^2), 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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