Glutathione Transferase (GSTM1) Null Genotype, Smoking, and Prevalence of Colorectal Adenomas

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Abstract

Colorectal cancer is caused by environmental exposures and genetic predisposition. However, little is known of hereditary factors that influence development of common, non-Mendelian forms of this cancer. Interactions among carcinogen exposure, hereditary variants of enzymes involved in carcinogen metabolism, and other host factors may play a role. Genetic polymorphisms of carcinogen metabolism, such as the glutathione transferase M1 (GSTM1) null genotype, are thus possibly related to cancer risk. The GSTM1 enzyme detoxifies mutagens formed from polycyclic aromatic hydrocarbons which are found in tobacco smoke. We analyzed GSTM1 genotypes and smoking among 488 controls and 446 individuals with a first-time diagnosis of colorectal adenomas which are precursors to cancer. Subjects were from two Kaiser Permanente sigmoidoscopy clinics in southern California. We observed no overall effect of the GSTM1 null genotype on the risk for colorectal adenomas (odds ratio, 0.85; 95% confidence interval = 0.65–1.10). The odds ratio for smokers with the null genotype was 2.07 (95% confidence interval = 1.14–3.77) when compared to “never smokers” without the null genotype. Using this same reference group, the odds ratio for smokers without the null genotype was 1.73 (95% confidence interval = 1.03–2.90). These two odds ratios were not significantly different (P = 0.30).

Introduction

Carcinogen-induced mutations in proto-oncogenes or tumor suppressor genes are involved in colorectal cancer. Many chemical carcinogens require enzymatic activation to become mutagenic, and detoxifying enzymes also exist. Interindividual variation in activity of such enzymes is recognized and may affect fates of carcinogens in vivo. Thus, interactions among carcinogen exposure, activation, and detoxification are apt to influence individual cancer risk. Polycyclic aromatic hydrocarbons are common carcinogens found in tobacco smoke, food, and combustion fumes; Ref. 1) whose mutagenic derivatives are detoxified by GSTM1.2 The GSTM1 null genotype produces complete lack of the GSTM1 enzyme due to homozygous deletion of the gene (2). The population frequency is 10–100%, depending on ethnic group (3, 4). The null genotype is associated with a higher risk for lung (5) and bladder cancer (6, 7) among smokers in some studies, but results are conflicting in others. Here we report analysis of the GSTM1 null genotype and cigarette smoking in a large, sigmoidoscopy-based, case-control study of colorectal adenomas, precursor lesions for cancer. We hypothesized that a GSTM1 null genotype effect would be larger in smokers due to polycyclic aromatic hydrocarbon exposure.

Patients and Methods

Study Population. Subjects were from either of two southern California Kaiser Permanente Medical Centers (Bellflower or Sunset) and had a sigmoidoscopy during the period from January 1, 1991, through August 25, 1993. Eligible men and women were 50–74 years old, fluent in English, and residents of Los Angeles County. Each had no history of invasive cancer, inflammatory bowel disease, familial polyposis, previous bowel surgery, severe gastrointestinal symptoms, or physical or mental disability that would preclude an interview. Cases had a first time diagnosis of one or more colorectal adenomas, confirmed by histology. Controls were selected from subjects who were free of polyps and were individually matched to cases by gender, age (within 5-year category), date of sigmoidoscopy (within 3-month category), and center attended.

We identified 628 potentially eligible cases and 689 potentially eligible controls during the accrual period. There were 70 cases and 94 controls who refused interviews, and we were unable to contact 29 cases and 32 controls. The response rate (number interviewed/number eligible) was thus 84% for cases and 82% for controls. When the control subject originally matched to a case could not be interviewed, a replacement was identified.

The indications for sigmoidoscopy among interviewed subjects were: (a) “routine” for 45% of cases and 44% of controls; (b) minor symptoms for 16%; (c) familial polyposis, inflammatory bowel disease, previous colorectal adenomas confirmed by histology, or a first-degree relative with colorectal cancer. The response rate was 76% for cases and 82% for controls.

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However, GSTM1 genotyping was performed on only 937 blood samples, because genotyping was discontinued roughly 2 weeks before collection of the last blood samples. Three participants were excluded due to missing data on ethnicity. The analyses were therefore based on data from 446 cases and 488 controls.

Laboratory Analysis. DNA was obtained from frozen buffy coat specimens. Three 50-μl drops of each specimen were placed on blotter paper and allowed to dry (Schleicher and Schuell No. 903). Use of dried blood spot DNA in a screening survey has been described elsewhere (9). The GSTM1 null genotype was detected by use of a PCR assay (10). The PCR assay was originally validated by concordance of PCR results with GSTM1 enzyme activities in peripheral white cells. However, because the GSTM1 gene is part of a family of 5 very similar genes, we further validated the assay by determining DNA sequences of PCR products from 6 individuals. Observed sequences matched the published sequence of GSTM1 (11), indicating that the assay is specific for this gene (data not shown). All PCR assays were done without knowledge of case or control status.

Data Analysis. Subjects homozygous for the GSTM1 gene deletion were coded as having the GSTM1 null genotype. Cigarette smoking data were abstracted from in-person interviews. Results of the main effect of smoking were presented elsewhere.* Individuals who had never smoked 100 cigarettes in their life were coded as never smokers. Subjects who had smoked more than 100 cigarettes were coded as ex-smokers if they quit smoking before the sigmoidoscopy and as current smokers if they were smoking at the time of sigmoidoscopy. Pack-years were estimated on the basis of the total number of years of smoking and on the average number of packs smoked/day. We categorized cases into those with adenomas <1 cm and ≥1 cm on the basis of the size of the largest adenoma.

Unmatched controls occurred when, e.g., their matched cases did not speak English or were found to have invasive cancer at follow-up colonoscopy. We used unconditional logistic regression to estimate odds ratios, in order to include information on these unmatched controls in the analysis. We controlled for the matching factors, date of sigmoidoscopy (6-month intervals), age (5-year intervals), gender, and center attended, as indicator variables in the model. An individual with a missing value on any covariate was excluded from analyses that included that variable.

Results and Discussion

The study population is described in Table 1. The overall odds ratio for adenomas with the GSTM1 null genotype was 0.85 (95% CI = 0.65–1.10), consistent with no overall GSTM1 effect. Stratification by ethnic group gave similar odds ratios (and 95% confidence intervals): Whites, 0.98 (0.69–1.40); Hispanics (not Black), 0.84 (0.43–1.66); Blacks, 0.61 (0.26–1.44); and Asians/Pacific Islanders, 0.43 (0.16–1.14).

Effects of smoking and GSTM1 genotype are shown in Table 2. Current smoking increased the risk of colorectal adenomas among subjects without the GSTM1 null genotype (OR = 1.73). The smoking effect was fairly comparable among subjects with the null genotype (OR = 2.07), compatible with no detectable GSTM1 effect (P = 0.30). In addition, lower cigarette use among cases with the GSTM1 null genotype (20.1 pack-years), compared to cases without the null genotype (23.6 pack-years; P = 0.27), was not statistically significant. Adjustment for potential confounders of the smoking effect (total energy intake; physical activity; body mass index; and intake of fruits and vegetables, saturated fat, alcohol, and nonsteroidal antiinflammatory drugs) did not substantially change the point estimates. For example, the adjusted odds ratio was 1.96 (95% CI = 1.04–3.68) for current smokers with the null genotype compared to those in the reference category. Subsequent analyses, therefore, included only the matching variables as covariates.

Information on adenoma size was available for all but 5 cases. The analysis was repeated for cases with adenomas 1 cm in size or larger (and controls). Instead of the values of 1.73 and 2.07 for current smokers (Table 2), values of 1.34 (95% CI = 0.61–2.92) and 2.45 (95% CI = 1.09–5.53) were obtained (P value for difference = 0.10).

Thus, there was a suggestion of a GSTM1 and smoking effect by adenoma size. Expanded studies are needed to address this question.

A link between smoking and colorectal neoplasms has often been shown by epidemiological studies. In prospective studies, the association with recent smoking was stronger for small adenomas than for large adenomas or colorectal cancer, whereas smoking in the more distant past seemed to increase the risk for large adenomas and cancer (12). The effect has been ascribed to dispersal of smoke mutagens to the colon via blood (13, 14), bile, or direct ingestion, although other mechanisms are possible (12).

Few data are available on the GSTM1 null genotype and colon neoplasia. One study compared GSTM1 genotypes in 196 colorectal cancer patients to genotypes in 225 controls (15). The frequency of the null genotype was higher among the patients, with odds ratios of 1.7 (P < 0.006) for distal colorectal cancers and 2.2 (P < 0.0001) for proximal cancers. The data support the conclusion of a higher risk for colorectal cancer with the GSTM1 null genotype. However, cases and controls in the study were from different hospital populations, and there was no assessment of smoking or potential confounding factors. Our results thus represent the first analysis of both GSTM1 and smoking in colorectal tumor development. We were unable to assess whether the GSTM1 null genotype affects the right colon more than the left, because only the left colon was accessible to the sigmoidoscope. Assuming that the etiologies of left- versus right-sided adenomas are potentially different, our study represents an unbiased analysis of left-sided adenomas.

A possible GSTM1 and smoking effect by adenoma size could reflect an influence on incidence and/or progression to larger tumors. Vogelstein et al. (16) found that “intermediate” adenomas (>1 cm) have K-ras mutations at a higher frequency (50 versus 10%) than early stage adenomas (≤1 cm). Because the GSTM1 gene is active in colon (17), lack of the gene could theoretically allow higher colon levels of some mutagens. Benzo(a)pyrene-diol-epoxide is a possible

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* Adjusted for date of sigmoidoscopy, age, gender, center attended, and ethnicity.

b Reference category.
example, whose metabolites circulate in vivo after inhalation (18) or ingestion (19).

In summary, the association of smoking with odds of colorectal adenomas was not modified by GSTM1 genotype in the 446 cases and 488 controls in our study. An odds ratio for adenomas 1 cm in size or smaller was somewhat higher, compatible with a possible GSTM1 effect. The data suggest that a GSTM1 null genotype effect, if any, is small. However, a high proportion of people lack the GSTM1 enzyme, and the prevalence of adenoma-bearing individuals over the age of 35 is also high (e.g., 12% in Norway; Ref. 20). Therefore, additional studies on the role of GSTM1 and smoking may be warranted.

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