CYP1A1 Genetic Polymorphisms and in Situ Colorectal Cancer

Lakshmi Sivaraman, Mary P. Leatham, Jonathan Yee, Lynne R. Wilkens, Alan F. Lau, and Loïc Le Marchand

Abstract
Numerous studies have associated colorectal adenoma with smoking and large bowel cancer with consumption of foods potentially containing polycyclic aromatic hydrocarbons. Enhanced metabolic activation of polycyclic aromatic hydrocarbons has recently been observed in homozygotes for a MspI mutation in the 3'-end of CYP1A1. We conducted a population-based case-control study to investigate whether CYP1A1 polymorphisms were related to colorectal cancer risk. Using polymerase chain reaction-based methods, we assessed the frequency of the MspI polymorphism in the 3'-end of CYP1A1 and another mutation in exon 7 of the gene (Ile-Val polymorphism) among 43 patients with in situ adenocarcinoma of the large bowel and 129 population controls. Homozygosity for the MspI mutant genotype was found to be positively associated with in situ colorectal cancer in Japanese (P = 0.008) and Hawaiians/partial-Hawaiians (P < 0.001), whereas the study lacked power to detect a similar association in Caucasians. The odds ratio for the homozygous variant genotype compared to the heterozygous and wild-type genotypes was 7.9 (95% confidence interval, 1.4—44.4) in Japanese. A similar association was suggested for the exon 7 mutation homozygosity in Japanese, as the two polymorphisms are in genetic disequilibrium. Thus, this study suggests a potentially important role for CYP1A1 and polycyclic aromatic hydrocarbons in the etiology of colorectal cancer in populations with a high gene frequency.

Introduction
Recent studies have provided strong evidence for an association of saturated fat and particularly red meat consumption with colorectal cancer (1). Particularly strong associations have been observed with consumption of broiled or grilled meats and browning of the meat surface (2). Exposure of foods to pyrolysis temperatures can lead to the formation of compounds, such as PAHs, heterocyclic amines, and others, that are carcinogenic in animals (3, 4). These compounds often require metabolic activation in order to exert their genotoxicity. Because wide interindividual variations in activity have been related to the existence of genetic polymorphisms for some of the activating enzymes, there is an opportunity to look for inherited metabolic susceptibilities to colorectal cancer. For example, the activation pathway for heterocyclic amines involves N-acetyltransferase (5), and individuals with the rapid acetylation phenotype have been found to be at increased risk for colorectal cancer (6).

In addition to diet, tobacco smoking may constitute another source of PAH exposure relevant to the large bowel. Smoking has consistently been associated with adenomatous polyps (7) and, more recently, with colorectal cancer (8). Thus, it is conceivable that PAHs play a role in some of the genetic alterations leading to colorectal tumor development.

CYP1A1 is of critical importance for the metabolism of PAHs. The gene product, AhH, initiates a multienzyme pathway that converts PAHs to their ultimate DNA-binding carcinogenic forms. A MspI RFLP of the 3'-end of the human CYP1A1 gene has recently been described which is in genetic disequilibrium with an adenine to guanine mutation at residue 462 in exon 7. The latter mutation causes an isoleucine to valine substitution in the heme binding region of CYP1A1, which appears to result in an increased enzymatic activity (9). These two polymorphisms have been shown to be more frequent in Japanese than in Caucasians and to be associated with lung cancer (10, 11). In the present study, we assessed these CYP1A1 polymorphisms in a population-based case-control study of in situ colorectal cancer and found that they may also be associated with large bowel cancer.

Materials and Methods

Materials. Chemicals were obtained from Sigma Chemical Company (St. Louis, MO). Proteinase K, MspI, and pGEM DNA markers were obtained from Promega Corporation (Madison, WI). AmpliTaq DNA polymerase was purchased from Perkin-Elmer Corporation (Norwalk, CT). 2'-Deoxyribonucleoside 5'-triphosphates were obtained from Pharmacia (Piscataway, NJ). Primers for PCR were synthesized by Ransom Hill BioScience, Inc. (Ramona, CA). Seakem ME and Seakem GTG agarose were obtained from FMC Bioproducts (Rockland, ME). HaeIII-digested 6X174 DNA molecular weight markers were from Gibco-BRL (Grand Island, NY).

Subjects. Participants are a subgroup of a larger case-control study of diet and colorectal cancer (12). For the present study, cases were all patients in one of the main medical centers on the island of Oahu, Hawaii, diagnosed with in situ adenocarcinoma of the large bowel between July 1989 and October 1991. By design, patients with invasive tumors were excluded because it was thought that advanced disease and its treatment would have affected some other biological parameters assessed in the study. Controls were identified among the participants in a population-based survey conducted by the Hawaii State Department of Health and were matched to each case on sex, age (within 5 years), and ethnicity. Overall, 72.4% of the eligible cases with in situ cancer and 71.6% of the eligible controls were interviewed. A total of 43 cases and 47 controls (or 73.4% of the eligible interviewed subjects) agreed to the biological component of the study and were available for the data analysis. Table 1 presents the sex and ethnic distributions of these participants.

In order to conduct race-specific analyses, we combined the controls described above with the controls from an ongoing, case-control study of genetic susceptibilities to lung cancer which uses an identical methodology (11). This additional series of controls genotyped for CYP1A1 included 37 Japanese, 26 Caucasians, and 14 Hawaiians.

DNA Extraction. Blood samples were analyzed blind to the case-control status of the subjects. DNA was extracted from peripheral blood lymphocytes by standard methods. The amount of DNA was determined spectrophotometrically, and its purity was estimated by comparing absorbances at 260 and 280 nm.

PCR-RFLP Analysis of 3'-End Polymorphism. Genotyping of the 3'-end polymorphism of the CYP1A1 gene (located at the 264th base downstream of the polyadenylation signal) was carried out by PCR amplification using primers 5'-TAGGAGTCCTTGCTCATGCCT-3' (C44) and 5'-CAGTGAAAGAG-

Received 4/12/94; accepted 6/2/94.

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The heterozygous genotype (type B) gave three bands at 340, 200, and 140 base pairs (Fig. 1a, Lane 1). The genotypes of the homozygous, rare mutant allele (type C), the wild-type 340-base pair undigested band at the 340-base pair position due to the absence of the MspI site, the template was heterozygous (A/G, Ile/Val).

Allele-specific PCR Detection of an A/G Polymorphism in Exon 7. The A→G transition polymorphism in exon 7 of the CYP1A1 gene was assessed by the allele-specific PCR method described by Hirvonen et al. (13). For this purpose, each of the primers 5'-AAGACCTCCACCGGCCGAAT-3' (wild-type 2A) and 5'-AAGACCTCCACCGGCCGCAAC-3' (mutant 2G) were used for PCR amplification together with the opposite strand primer 5'-GAAAGGCTGGGTCCCACCCTCTC-3' (primer 1) whose 5'-end is located 303 base pairs upstream of the A/G polymorphic site. PCR products were then subjected to electrophoresis in 1.8% Seakem ME agarose gel.

Statistical Analyses. $\chi^2$ was used to test for case-control differences in the distributions of the genotypes or other parameters studied. ORs and 95% CIs were computed to compare risk between levels of categorical variables. Logistic regression was used to estimate ORs when adjusting for covariates. The t test was used for testing differences in continuous variables.

Results

Controls were similar to cases with regard to sex, age, ethnicity, education, body size, and smoking and drinking habits (Table 1). However, cases were somewhat more likely to report a family history of colorectal cancer among first degree relatives ($P = 0.15$).

The MspI RFLP analysis is illustrated in Fig. 1a. DNA templates of genotypes A, B, and C were PCR amplified as described in "Materials and Methods," and the amplified product was digested with MspI. The fragment amplified from type A (wild-type) DNA gave a single, undigested band at the 340-base pair position due to the absence of the restriction site (Fig. 1a, Lane 3). When the template represented a homozygous, rare mutant allele (type C), the wild-type 340-base pair fragment was cleaved into 200- and 140-base pair bands (Fig. 1a, Lane 2). The heterozygous genotype (type B) gave three bands at 340, 200, and 140 base pairs (Fig. 1a, Lane 1). The genotypes of the CYP1A1 gene ascribed to the MspI site as identified by RFLPs and PCR were in complete agreement with those obtained by Southern blotting analysis (data not shown).

Fig. 1b presents the results of a typical allele-specific PCR reaction used to identify the A→G transition mutation at position 462 of AHH which results in an isoelucine to valine amino acid substitution. The primers were designed so that the 3' terminal position precisely matched either the wild-type "A" allele (primer 2A) or the mutant "G" allele (primer 2G). The wild-type homozygote (A/A, Ile/Ile) produced a product of 322 base pairs with the combination of primers 1 and 2A (Fig. 1b, Lane 2A). The combination of primers 1 and 2G gave no PCR product due to the 3'-end mismatched base pair (Fig. 1b, Lane 2G). Conversely, a homozygous mutant template (G/G, Val/Val) gave the 322-base pair product with the combination of primers 1 and 2G (Fig. 1b, Lane 2G) but not with the combination of primers 1 and 2A (Fig. 1b, Lane 3A). A product appeared with a combination of both primer pairs 1 and 2A and 1 and 2G (Fig. 1b, Lanes 1A and 1G) when the template was heterozygous (A/G, Ile/Val).

The genotypes for the exon 7 point mutation in the coding region were correlated ($\phi = 0.69; P < 0.001$) with those for the MspI mutation in the noncoding region, suggesting that the two polymorphisms are closely linked as previously described in Japanese (9) and Northern Europeans (13).

The frequencies of the CYP1A1 polymorphisms among cases and controls are shown in Table 2. Cases were more likely to be homozygous for the MspI variant genotype ($P = 0.008$). The OR and 95% CI for the C genotype compared to the two other genotypes was 6.8 (1.4–33.1). Adjustment for age and sex by logistic regression did not modify this risk estimate. There was no increased risk associated with the heterozygous variant genotype compared to the A genotype. The homozygous genotype for the A→G mutation on exon 7 was also more frequent in cases than controls, although with our current limited sample size, this difference did not quite reach statistical significance ($P = 0.14$, comparing G/G to the two other genotypes).

Table 3 presents the ethnic-specific analysis using the expanded control group. The distribution of the MspI genotypes in the Japanese controls (26, 31, and 2 subjects for the A, B, and C genotype, respectively) is consistent with the Hardy-Weinberg equilibrium for Mendelian inheritance ($P = 0.35$). The frequency of the MspI variant allele was found to be greater in Hawaiians (0.38) and Japanese (0.30) than in Caucasians (0.09). As expected from past studies (9, 13), the Ile-Val polymorphism was less common than the MspI polymorphism, but its frequency followed a similar pattern among the ethnic groups studied.

A statistically significant association between in situ colorectal cancer and the homozygous MspI mutant genotype was found in both Japanese ($P = 0.008$) and Hawaiians ($P < 0.001$; Table 3), whereas the study had only minimal power to detect an association in Caucasians due to their lower gene frequency (0.09). The OR and 95% CI for the C genotype compared to the other genotypes was 7.9 (1.4–44.4) in Japanese. There was also the suggestion of an association between colorectal cancer and the homozygous Ile-Val mutant genotype in Japanese ($P = 0.12$) with an OR of 5.7 and a 95% CI that included 1.0. The Ile-Val polymorphism was absent from our Caucasian ($n = 41$) and Hawaiian ($n = 26$) samples.

Discussion

To our knowledge, this is the first report of an association of CYP1A1 genetic polymorphisms with colorectal cancer risk. In this population-based study, we found an association between in situ large bowel cancer and homozygosity for the MspI RFLP which was strong (OR = 7), statistically significant ($P = 0.008$), and present in two of the three ethnic groups studied. A similar association was suggested for the Ile-Val polymorphism to which the MspI RFLP is closely linked (9, 13), adding to the internal consistency of the data. However,
Table 2 CYPIAI genotype frequencies in situ colorectal cancer cases and controls

<table>
<thead>
<tr>
<th>Marker</th>
<th>Genotype</th>
<th>Cases (n = 43)</th>
<th>Controls (n = 47)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mspl</td>
<td>A</td>
<td>0.53</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.23</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.23</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P = 0.008</td>
<td></td>
</tr>
<tr>
<td>Ile-Val</td>
<td>A/A</td>
<td>0.74</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>A/G</td>
<td>0.21</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>0.05</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P = 0.23</td>
<td></td>
</tr>
</tbody>
</table>

because these findings are based on relatively small numbers of cases, they need replication.

The frequencies for the Mspl polymorphism in our population are similar to those described for Japanese and Caucasians elsewhere. Kawajiri et al. (15) in Japan reported a frequency of 33% for the Mspl variant allele in Japanese compared to 30% in our study. In contrast, the gene frequency for Caucasians was reported to be 14% in Maryland (16) and 12% in Finland (13) and Norway (17) compared to 9% in the present study. Our finding of a high prevalence for the Mspl variant allele in Hawaiian Polynesians (38%) is consistent with the origination of the Polynesians from Southeast Asia and the frequent Oriental admixture of today's Hawaiians. We are not aware of any other studies of CYPIAI polymorphism in other Polynesian populations.

The Mspl RFLP has been shown to cosegregate with the high AHH inducibility phenotype in a family study (18) and to correlate with enhanced enzymatic activity in lymphocytes from unrelated individuals (19). Thus, homozygotes for the variant allele are expected to be at a greater cancer risk when exposed to PAHs. Indeed, Japanese smokers with this genotype have been shown to have a 3-fold increase in risk for lung cancer (10). No such association has been reported for Caucasians, although their lower gene frequency may have limited the statistical power of past studies.

PAHs are known to be present in charcoal-broiled and barbecued meat, as well as in a variety of smoked foods and in roasted coffee (20). These foods have all been associated with colorectal cancer with some consistency in past studies (20). Tobacco smoking may constitute another source of PAH exposure for the large bowel through the circulation or direct ingestion. Smoking has consistently been associated with adeno-
mas (7) and, more recently, with colorectal cancer only after allowing for a long induction period (8), suggesting a role for PAHs and/or other carcinogens in tobacco smoke in the early genetic alterations leading to colorectal tumors. If PAHs from tobacco smoke are predominantly involved, it may be that, as it has been the case for smoking, an association with the CYP1A1 polymorphisms will be more difficult to replicate for invasive tumors than for adenomas.

PAHs have not been found to be effective colorectal carcinogens in animals, except in some inbred strains of hamsters for which oral administration resulted in large bowel carcinomas (21). PAH metabolism has been described to take place in the colon and to result in the formation of DNA adducts as in several other tissues (22). More specifically, PAH metabolites have been shown to activate the H-ras protooncogene in vivo (23), a gene closely related to K-ras, which is found to be mutated in a number of human colorectal cancers in the United States (24) and which is thought to be altered in early in the progression from adenoma to carcinoma (25). PAH metabolites are detoxified by GSTφ, and individuals with the null GSTM1 genotype have been shown to have a 3-fold greater risk of adenocarcinoma of the colon and stomach (26).

Given the present finding of an association with the homozygous variant MspI polymorphism of the CYP1A1 gene, PAHs may be important etiological agents in colorectal cancer, particularly in populations with a high gene frequency. Future studies need to investigate the combined etiological roles of smoking, dietary exposures, and polymorphic genes involved in carcinogen metabolism (e.g., CYP1A1, NAT2, and GSTM1) for both colorectal adenomas and carcinomas.

Acknowledgments

We thank Castle Medical Center, Kaiser-Permanente Medical Center, Kuakini Medical Center, Queen's Medical Center, Straub Clinic and Hospital, St. Francis Medical Center, Tripler Medical Center, and Wahiawa General Hospital for their support of this study.

References


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