Bile acids have long been implicated in the etiology of colorectal cancer, but epidemiologic evidence remains elusive. Cholesterol 7α-hydroxylase (CYP7A1) is the rate-limiting enzyme in the synthesis of bile acids from cholesterol in the liver, and thus may be an important determinant of bile acid production. We examined the association between the CYP7A1 A-203C polymorphism and colorectal cancer. The CYP7A1 A-203C polymorphism was determined by the PCR-RFLP method in 685 incident cases of colorectal cancer and 778 controls randomly selected from a community in the Fukuoka area, Japan. The CC genotype was slightly less frequent in the case group, and the adjusted odds ratio for the CC versus AA genotype was 0.88 (95% confidence interval, 0.65-1.20). In the analysis by subsite of the colorectum, a decreased risk associated with the CYP7A1 CC genotype was observed for proximal colon cancer, but not for either distal colon or rectal cancer. The adjusted odds ratios (95% confidence intervals) of proximal colon cancer for the CC genotype were 0.63 (0.36-1.0) compared with the AA genotype, and 0.59 (0.37-0.96) compared with the AC genotype combined. A decreased risk of proximal colon cancer in relation to the CC genotype of CYP7A1 A-203C, which probably renders less activity of the enzyme converting cholesterol to bile acids, is new evidence for the role of bile acids in colorectal carcinogenesis. (Cancer Res 2005; 65(7): 2979-82)

Introduction

Colorectal cancer is one of the most common cancers in the world, accounting for nearly 10% of all incident cases of cancer (1). Japan has experienced a rapid increase in mortality from colorectal cancer in the past 50 years (2), and is currently among the countries with the highest incidence rates worldwide (3). Bile acids have long been implicated in the etiology of colorectal cancer. Primary bile acids such as cholic and chenodeoxycholic acids are excreted in the liver, and are degraded to secondary bile acids, mainly deoxycholic and lithocholic acids, by bacteria in the intestinal lumen. Animal studies showed that secondary bile acids promoted chemically induced colorectal cancer (4, 5), and recent in vitro studies have identified several molecular mechanisms of deoxycholic acid promoting colorectal carcinogenesis (6, 7).

Despite these experimental observations, epidemiologic evidence remains elusive regarding the role of bile acids in colorectal carcinogenesis. Fecal levels of secondary bile acids as well as of total bile acids are higher in populations at high risk of colorectal cancer (8, 9). Several case-control studies reported higher levels of secondary bile acids in the feces or sera in patients with colorectal cancer or adenomas as compared with those without these lesions (10-13), but the findings were not replicated in other studies (14-16). A prospective study reported a suggestive increase in the risk of colorectal cancer associated with a high ratio of serum deoxycholic to cholic acids (17). Another epidemiologic evidence is the increased risk of proximal colon cancer in individuals having the gallbladder removed (18, 19). Cholecystectomy results in increased fecal excretion of secondary bile acids, probably due to increase in the bile acid pool in the enterohepatic circulation and increased degradation of primary bile acids in the gut (20, 21).

Recent studies (22, 23), but not all (24), showed that a common genetic polymorphism of cholesterol 7α-hydroxylase (CYP7A1 A-203C) was associated with plasma total and low-density lipoprotein cholesterol concentrations, suggesting lower activity of the enzyme in individuals with the variant C allele. CYP7A1 is the rate-limiting enzyme in the synthesis of bile acids from cholesterol in the liver, and thus may be an important determinant of not only plasma cholesterol levels but also bile acid production. This article examined the association between the CYP7A1 A-203C polymorphism and colorectal cancer in order to further clarify the role of bile acids in colorectal carcinogenesis.

Materials and Methods

A case-control study was designed to examine the relation of lifestyle factors and genetic susceptibility to the risk of colorectal cancer. Cases were recruited from eight large hospitals in the study area (Fukuoka City and three adjacent areas), and controls were randomly selected in the community by frequency-matching to the distribution of incident cases with respect to sex and 10-year age class. The study protocol was approved.

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by the ethical committees of the Faculty of Medical Sciences, Kyushu University, and of all but two of the participating hospitals. The two hospitals had no ethical committees at the time of survey. Details of the methods have been described elsewhere (25).

Participants. Cases were a consecutive series of patients with histologically confirmed incident colorectal adenocarcinomas who were admitted to two university hospitals or six affiliated hospitals for surgical treatment during the period October 2000 to December 2003. Other eligibility criteria were: age of 20 to 74 years at the time of diagnosis, residence in the study area, no prior history of partial or total removal of the colorectum, familial adenomatous polyposis or inflammatory bowel disease, mental competence to give informed consent and to complete the interview. Of 1,053 eligible cases, a total of 840 cases (80%) participated in the interview, and 685 out of them gave an informed consent to genotyping.

Eligibility criteria for controls were the same as described for cases except for the two items, i.e., having no diagnosis of colorectal cancer and age of 20 to 74 years at the time of selection. A total of 1,500 persons were selected as control candidates by two-stage random sampling. The number of control candidates by sex and 10-year age class were determined in accordance to sex- and age-specific numbers of estimated incident cases of colorectal cancer. The first step was a random selection of 15 small areas out of 178 in total, and then 100 persons were randomly selected in each small area using the municipal resident registry on the basis of proportions of population in the small areas by sex and 10-year age class. A letter of invitation was sent to each candidate, and at most three additional letters of invitation were mailed to nonrespondents. A total of 833 persons participated in the survey, and 778 gave an informed consent to genotyping.

The net participation rate was calculated as 60% (833/1,382), which is defined as the ratio of the number of participants to the total number of invitees. A letter of invitation was sent to each candidate, and at most three additional letters of invitation were mailed to non-responders. A total of 833 persons participated in the survey, and 778 gave an informed consent to genotyping. The net participation rate was calculated as 60% (833/1,382), after exclusion of 118 persons for the following reasons: death (n = 7), migration from the study area (n = 22), undelivered mail (n = 44), mental incompetence (n = 19), history of partial or total removal of the colorectum (n = 21), and diagnosis of colorectal cancer after the survey (n = 5).

In both cases and controls, older persons and women were less likely to give consent to genotyping, whereas there was no material difference in residence, smoking habit, and alcohol use between individuals giving consent and those who did not (Table 1).

Procedures. DNA was extracted from the buffy coat by using a commercial kit (Qiagen GmbH, Hilden, Germany). Genotyping was done by one of the authors (T. Hagiwara) using the PCR-RFLP method. The PCR was done in a reaction mixture of 10 μL containing 0.5 units of Taq and 1 μL of template DNA with a concentration of 50 to 150 ng/μL. The CYP7A1 genotype was determined, as described by Han et al. (24) using primers 5′-AATGT TTTTC CCAGT TCTCT TTG-3′ (sense) and 5′-ATTTA GCCAT TTGTT CATTC TATTA G-3′ (antisense). After the initial denaturation at 94°C for 4 minutes, 30 cycles of PCR were done for 30 seconds at 94°C, for 30 seconds at 53°C, and for 30 seconds at 72°C, with a final extension at 72°C for 7 minutes. The PCR product of 393 bp fragment was digested with 10 units of BsaI in a reaction mixture of 20 μL for 3 hours at 50°C. The digestion results in fragments of 300 and 93 bp for the A allele, and those of 261, 93, and 39 bp for the C allele. The digested PCR products were applied to electrophoresis of 3% agarose gel (NuSieve GTG, Rockland, ME), and visualized by ethidium bromide.

The polymorphism was referred to as A-203C by Couture et al. (23), but the actual site of the polymorphism is located 203 bp upstream of the transcription start site according to the latest report of the sequence http://www.ncbi.nlm.nih.gov/Genomes. This was also confirmed by our sequencing of the relevant fragment.

Statistical analysis. The association of CYP7A1 genotypes with the risk of colorectal cancer was examined in terms of odds ratio (OR) and 95% confidence intervals (CI). ORs were obtained from multiple logistic regression analysis, including indicator variables for gender, 5-year age class, and residence area (Fukuoka City or suburban area) as covariates. Statistical significance was declared if 95% CI did not include unity. All statistical analyses were done using the SAS version 8.2 (SAS Institute, Inc., Cary, NC).

Results

Proportions of the AA, AC, and CC genotypes in cases of colorectal cancer were 24%, 56%, and 20%, respectively (Table 2). The corresponding proportions in the control group were 25%, 51%, and 24%, respectively. The distribution in the control group was in agreement with the Hardy-Weinberg equilibrium (P = 0.59). The CC genotype was slightly less frequent in the case group, and the adjusted OR for the CC versus AA genotype was slightly lower than unity, with the 95% CI including unity. When the AA and AC genotypes were combined as the referent, the adjusted OR for the CC genotype was 0.81 (95% CI, 0.63-1.04).

The association with CYP7A1 polymorphism was further examined for cancers of the proximal colon, distal colon, and rectum separately (Table 3). A nearly significant decrease in the OR for the CC versus AA genotype was observed for proximal colon cancer, but not for the other sites of cancer. When the AA and AC genotypes were combined as referents, the adjusted ORs of proximal colon cancer for the CC genotype was significantly lower than unity.

Discussion

The present study was the first that examined the relation between a functional CYP7A1 polymorphism (A-203C) and...
colorectal cancer, and showed a decreased risk of cancer of the proximal colon, but not of the distal colon and rectum, among individuals having the CC genotype. This genotype is probably associated with lowered capability of synthesizing bile acids (22, 23), the findings provide further evidence to the role of bile acids in colorectal carcinogenesis.

An advantage in this large-scale case-control study is that controls were derived from free-living residents in the community. It is also notable that participation rates of eligible cases and controls were fairly high. Genotyping was done in 82% of the cases and 93% of the controls who participated in the survey. It is, however, possible that use of hospital controls may cause selection bias even in the gene-disease association. For instance, individuals with high blood cholesterol levels may have been included or excluded differentially in the controls if selection had occurred in patients with cholesterol-related diseases. The study subjects were an ethnically homogenous population of Japanese, and the concern over population stratification would be negligible (28).

Since the first report by Rose et al. (29), many prospective studies have observed an inverse association between serum total cholesterol and colon cancer (30). Although this inverse association is generally ascribed to the effect of preclinical cancer existing at the baseline (30), an increased risk of proximal colon cancer associated with low levels of serum total cholesterol persisted 10 to 20 years later in a prospective study in Hawaii (31). Furthermore, a case-control study observed lower levels of total and low-density lipoprotein cholesterol in cases of proximal colon cancer, but not of distal colon cancer, than in controls (32). These findings are congruent with decreased risk of proximal colon cancer associated with the CYP7A1 CC genotype.

High-fat diets are shown to increase fecal excretion of secondary bile acids as well as of total bile acids in humans (33), and to enhance chemically induced colon carcinogenesis in animals (34). Although fat intake is strongly positively correlated with colon cancer rates among countries (35), and over time in Japan (36), studies of individuals have consistently failed to find a positive association between fat intake and colon or colorectal cancer (37). The lack of an association with fat in studies of individuals may be due to a limited variation of fat intake within populations. In this regard, the present findings emphasize the usefulness of studying functional genetic polymorphisms when study populations are homogeneous with respect to exposure to environmental factors such as nutrient and food intake.

In conclusion, a large case-control study in Japan showed a decreased risk of proximal colon cancer in individuals having the CC genotype of CYP7A1 A-203C, which probably renders less activity of the enzyme converting cholesterol to bile acids. The findings add to evidence for the role of bile acids in colorectal carcinogenesis.

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**Table 2. Adjusted ORs and 95% CI of colorectal cancer according to CYP7A1 A-203C polymorphisms**

<table>
<thead>
<tr>
<th>CYP7A1 A-203C genotype</th>
<th>Number (%)</th>
<th>Cases (n = 685)</th>
<th>Controls (n = 778)</th>
<th>Adjusted ORs (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>163 (24)</td>
<td>193 (25)</td>
<td>1.00 (referent)</td>
<td></td>
</tr>
<tr>
<td>AC</td>
<td>385 (56)</td>
<td>399 (51)</td>
<td>1.13 (0.88-1.46)</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>137 (20)</td>
<td>186 (24)</td>
<td>0.88 (0.65-1.20)</td>
<td></td>
</tr>
</tbody>
</table>

*Adjusted for gender, 5-year age class, and resident area.

**Table 3. Adjusted OR and 95% CI of colorectal cancer according to CYP7A1 A-203C genotypes by subsite**

<table>
<thead>
<tr>
<th>CYP7A1 A-203C genotype</th>
<th>Proximal colon (n = 150)</th>
<th>Distal colon (n = 232)</th>
<th>Rectum (n = 290)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>OR (95% CI)*</td>
<td>No.</td>
</tr>
<tr>
<td>Model 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>39</td>
<td>1.00 (referent)</td>
<td>52</td>
</tr>
<tr>
<td>AC</td>
<td>88</td>
<td>1.09 (0.71-1.65)</td>
<td>129</td>
</tr>
<tr>
<td>CC</td>
<td>23</td>
<td>0.63 (0.36-1.10)</td>
<td>51</td>
</tr>
<tr>
<td>Model 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA + AC</td>
<td>127</td>
<td>1.00 (referent)</td>
<td>181</td>
</tr>
<tr>
<td>CC</td>
<td>23</td>
<td>0.59 (0.37-0.96)</td>
<td>51</td>
</tr>
</tbody>
</table>

*Adjusted for gender, 5-year age class, and resident area.
References


Genetic Polymorphism in Cytochrome P450 7A1 and Risk of Colorectal Cancer: The Fukuoka Colorectal Cancer Study

Tomoko Hagiwara, Suminori Kono, Guang Yin, et al.


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