A Molecular Variant of the APC Gene at Codon 1822: Its Association with Diet, Lifestyle, and Risk of Colon Cancer

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ABSTRACT

The adenomatous polyposis coli (APC) gene is important in the etiology of colon cancer. Although germ-line mutations of this gene rarely occur in the population, less penetrant variants of the gene have been reported. One variant, producing an aspartate to valine change at codon 1822 (B1822V) has been previously reported as having an allele frequency of 10%. The purpose of this study was to determine whether this B1822V variant of the APC gene is associated with colon cancer and whether its association is influenced by other genetic or environmental factors. We used data collected as part of a multicenter study of 1585 incident cases of colon cancer and 1945 age- and sex-matched population-based controls to evaluate genetic, dietary, and environmental associations with the B1822V variant of the APC gene. The frequency of the valine/valine allele at codon 1822 was 22.8% in this population. In the control population, 61.5% were homozygote wild type, 33.3% were heterozygotes, and 5.2% were homozygote variant. Cases were slightly less likely to have the homozygous variant APC genotype than were controls [odds ratio (OR), 0.8; 95% confidence interval (CI), 0.6–1.1]; for those diagnosed after age 65, the homozygous APC variant was associated with reduced risk of colon cancer (OR, 0.6; 95% CI, 0.4–1.0). Assessment of the homozygous APC variant with dietary, genetic, and environmental factors showed that individuals with this genotype were at lower risk if they consumed a low-fat diet (OR, 0.2; 95% CI, 0.1–0.5) relative to those who were homozygous wild type and ate a high-fat diet. This finding was specific to a low-fat diet and was unrelated to other dietary variables. These results suggest that the codon 1822 variant of the APC gene may have functional significance. Individuals who have the valine/valine variant of this gene may be at reduced risk of colon cancer if they eat a low-fat diet.

INTRODUCTION

The APC gene is part of the wnt signaling pathway and important in the etiology of colon cancer (1, 2). Acquired mutations in this gene are important early steps in the carcinogenesis process, with ~80% of sporadic colon tumors showing such a mutation (3). Although acquired mutations occur throughout the gene, there is a mutation cluster region at codons 1286 through 1513 in sporadic cancers. Germ-line variants of the APC gene exist (4). The best known of these are the relatively uncommon inherited knockout mutations which are responsible for the syndrome of Familial Adenomatous Polyposis. It is estimated that fewer than 1% of colon cancers can be attributed to these highly penetrant inherited APC mutations (5). There are, however, other germ-line variants that are not associated with the polyposis phenotype and are of uncertain biological significance (3). At least eight molecular variants of the APC gene have been identified. With the exception of one variant, the allele frequencies of these variants are estimated at no more than 1% (3, 6, 7). Three of these variants have been associated with amino acid changes (3). The most common variant has been reported at an allele frequency of 10%; it results in an aspartate to valine change at codon 1822 (3). The other two variants causing amino acid changes have been reported with allele frequencies of around 1% (3).

It is possible that APC variants associated with amino acid changes may alter risk of colon cancer in the presence of environmental exposures, or that they may independently alter colon cancer risk in subsets of the population. In one study by Powell et al. (3), significant associations between colon cancer and the three APC variants that cause amino acid changes were not detected in 45 cases of cancer and 100 healthy controls; that study had limited power.

In this study, we used data from a large population-based case-control study of colon cancer to examine the association between the codon 1822 variant of the APC gene and risk of colon cancer. We also determined whether dietary and life-style exposures influenced colon cancer risk in conjunction with this APC variant. Because of its relatively high allele frequency (3), we projected that we would have sufficient power to detect an approximate 2-fold difference in risk associated with diet and life-style factors that might interact with the B1822V variant.

PATIENTS AND METHODS

Study Population. Study participants were Caucasian (91.3%), African-American (4.2%), or Hispanic (4.4%) and from the Kaiser Permanente Medical Care Program of Northern California (KPMCP), an eight county area in Utah (Davis, Salt Lake, Utah, Weber, Wasatch, Tooele, Morgan, and Summit counties) and the metropolitan Twin Cities area (Anoka, Carver, Dakota, Hennepin, Ramsey, Scott, and Washington counties) of Minnesota. A rapid-reporting system was used to identify cases, with the majority of cases being interviewed within 4 months of diagnosis. Eligibility criteria for cases included diagnosis with first-primary incident colon cancer (ICD-O 2nd edition (8) codes 18.0 and 18.2 to 18.9) between October 1, 1991 and September 30, 1994; ages between 30 and 79 years at time of diagnosis; and mental competence to complete the interview. Patients with cancers of the rectosigmoid junction or rectum (defined as the first 15 cm from the anal opening), with known familial adenomatous polyposis, ulcerative colitis, or Crohn’s disease were not eligible. Of those cases invited to participate in the study, ~76% cooperated. Methods used to ascertain controls have been reported and included people randomly selected from Kaiser membership lists, from random-digit dialing, from driver’s license lists, and from social security lists (9). Of all controls asked to participate, ~64% cooperated. Reasons for nonparticipation have been described (10). A total of 1993 cases and 2410 controls had complete data considered of high quality. Of these, 1585 cases and 1945 controls had DNA

Received 6/15/00; accepted 11/29/00.
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1 Supported by Grant CA48998 (to M. L. S.) and Grant CA93045 (to J. D. P.). Case identification and verification were supported by the Utah Cancer Registry, the Northern California Cancer Registry, and the Sacramento Tumor Registry.
2 To whom requests for reprints should be addressed, at Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue, North, MP 900, Seattle, WA 98109-1024.
3 The abbreviations used are: APC, adenomatous polyposis coli; OR, odds ratio; CI, confidence interval; NSAID, nonsteroidal anti-inflammatory drug; BMI, body mass index; DAG, diacylglycerol.
Dietary intake data were ascertained using an adaptation of the validated Coronary Artery Risk Development in Young Adults (CARDIA) diet history questionnaire (12). Participants were asked to determine which foods were eaten (using brand names of food items such as fast foods, cookies, crackers, cereals, when possible), the frequency with which foods were eaten, and fat used in preparation of other foods. Three-dimensional food models were used to help participants estimate their usual serving size. Cue cards were used to help the consistent identification of foods within broad categories. For certain items, when it was possible that many types of food within a category would be eaten (such as breakfast cereal), participants were asked to report the three mostly commonly eaten items. As part of the diet history, detailed information was obtained on foods eaten as additions to other foods (such as sugar added to cereal); standard amounts of additions were assigned per unit of the food item those accompanied. Nutrient values for specific foods were calculated using the Minnesota Nutrition Coordinating Center’s (NCC) nutrient database version 19.

Specific questions on the preparation of red meat, poultry, and fish were used including those on the preferred degree of cooking (“doneness”) of red meat and poultry: rare, medium rare, medium well done, and well done; the frequency of cooking by frying, broiling, baking, or barbecuing of red meat, poultry, and fish; and the frequency of the use of drippings of red meat, poultry, and fish, either on other foods or in gravy. Because microwaving produces much lower concentrations of mutagens than pan frying and broiling, the frequency of use of microwaving to thaw or partially precook meat before preparation by frying, broiling, baking, or barbecuing was assessed. We estimated potential exposure to mutagens via a Mutagen Index. The index is calculated as [the frequency of red meat, poultry, and fish consumption prepared by frying, broiling, baking, or barbecuing] plus [the use of drippings from red meat, poultry or fish] × [the preferred doneness of the red meat, poultry, and fish (1 = rare, 2 = medium rare, 3 = medium well done, 4 = well done)] × [the microwave factor (1 = never used for thawing, 0.75 = sometimes used, 0.5 = often used, 0.25 = always used)]. A high index reflects higher intake of potentially mutagenic compounds.

Measures of eating patterns were derived using factor analysis, as described elsewhere (13) via the SAS principal-components program. After a varimax rotation, factor scores were saved for each individual. The food pattern that we arbitrarily labeled as “Western Diet” was used for the analyses presented here. This food pattern loaded heavily (factors with loadings of over 0.30) on processed meats, red meat, fast-food meat, eggs, butter, margarine, potatoes, high fat dairy foods, legumes, refined grains, added sugar, sugar drinks, and sugar desserts (13).

Other data obtained and used in these analyses were: age at the time of diagnosis or selection; BMI (weight/height²) for men; weight/height³ for women) self-reported for the referent year; usual number of cigarettes smoked, long-term vigorous leisure-time activity, ever having used aspirin or non-steroidal anti-inflammatory drugs on a regular basis, and family history of cancer in first-degree relatives. Physical activity, performed at home and at leisure, was ascertained using an adaptation of the validated CARDIA physical activity history (14). Tumor site within the colon was classified as proximal (cecum through transverse colon) or distal (splenic flexure, descending, and sigmoid colon). Tumor stage was defined using the NCI’s Surveillance Epidemiology and End Results (SEER) Program summary stage codes of local, regional or distant. Vital status was obtained from local tumor registries and included all deaths through December of 1998.

Genotype Data. The GAC/GTC polymorphism at codon 1822 of the APC gene (6) was determined for 1590 cases and 1945 controls by single-strand conformational polymorphism. This was done as described previously (15) except that primers were fluorescently tagged, and PCR products were run on a PE Biosystems 373A sequencer. A segment of DNA that contained codon 1822 was PCR amplified using a forward primer fluorescently tagged with TET (GTTCCTCAGAGATTTTTCTCAGAAC) and one of six reverse primers (GTTCCTCATCTCATATTTTGGGAGG, GTTCCTCTATCTC- TATATTGGGAGG, GTTCCTACTTCTCTGACTCTATCTC, GTT- TCAAGATCAAAGCAAACTTCTC, GTTCCTATGAAGTTGCAAA- CAAACCA, GTTCCTTCAGGCTGATGATGAGTGGT, or GTTCCTT- CCTTCAATAGGCGTGTAAAG). The six reverse primers were used to generate PCR products of different lengths so that these products could be multiplexed on the 373A sequencer. An identical forward primer tagged with HEX was used to amplify a known normal to provide an internal control in each lane (Fig. 1).

Samples were amplified in a 20-µl reaction consisting of 10 mM Tris-HCl, 1.5 mM MgCl₂, 200 μM each dNTP, 0.5 μM each primer, and 0.25U Taq polymerase. The thermal cycling profile was 5-min initial denaturing at 95°C, 8 cycles of (20 s at 95°C, 20 s at 62°C, 40 s at 72°C), decreasing the annealing temperature 1° per cycle), 20 cycles of (20 s at 95°C, 20 s at 54°C, 40 s at 72°C), and a final 10 min extension at 72°C. Samples were prepared with loading buffer, denatured, and loaded on a 12-cm wr Mutation Detection Enhancement gel (from FMC) as described in the PE Biosystems GeneScan Reference Guide section 7. The gel was run on a PE Biosystems 373S genotyper at 10 W for 9 h and analyzed with GeneScan and Genotyper software.

Statistical Methods. APC B1822V genotype was classified as the homozygous wild type, heterozygote, or the homozygous variant. The APC genotypes were assessed in conjunction with demographic factors such as age at time of diagnosis, disease stage, tumor site within the colon, and survival status. Multiple logistic regression models were used to estimate associations using ORs and corresponding 95% CIs. Continuous diet and life-style factors were categorized into three groups of low, intermediate, and high, based on the sex-specific distributions in the control population. Nutrients were assessed as intake per 1000 kcal. Cigarette smoking was categorized as never having smoked cigarettes, smoking fewer than 20 cigarettes per day, and smoking 20 cigarettes per day or more. Regular use of aspirin and/or NSAIDs (combined and referred to as NSAIDs) and having a first-degree relative with colorectal cancer were categorized as either yes or no. Risk was assessed by examining the combined effects of B1822V genotype, diet, and life-style exposures, using as the referent category, those at greatest risk, e.g., high dietary fat and homozygote wild-type APC.

RESULTS

The B1822V allele frequency was 22.8% in the control population; the genotypes were in Hardy-Weinberg equilibrium. Homozygous-variant APC 1822 (valine/valine) individuals accounted for ~5.2% of the control population (Table 1). There were no statistically significant differences in the proportion of cases and controls with this genotype by sex or race, stage of tumor at diagnosis, tumor site within the colon, or the likelihood of dying up to 9 years after diagnosis (data not shown). The homozygous APC variant genotype did appear to have an association with age; cases diagnosed at an older age were statistically significantly less likely to have this genotype than age-matched controls.

Assessment of dietary factors and APC 1822 genotypes, showed a
consistent pattern of association with dietary fat variables (Table 2); adjustment of each of the specific subtypes of fat for total fat did not alter these associations. Relative to homozygous wild-type individuals and a high-fat diet, those who were homozygous variant with a low-fat diet had a markedly lower colon cancer risk. This reduction in risk was not observed for low-fat diet among homozygous wild type or heterozygotes. Ways of assessing diet (categorized or continuous) or grouping genotypes (collapsing homozygous wild type or heterozygotes). Other alterations may have been at a slightly less elevated risk of colon cancer if they also carried the homozygous variant APC genotype.

**DISCUSSION**

Although acquired mutations in the APC gene are commonly observed in colon tumors, inherited mutations causing familial adenomatous polyposis are rare. However, it is possible that germ-line variants of the APC gene that result in amino acid changes may have functional significance, especially in the presence of diet, life-style, or other environmental exposures. In this study, we have characterized the most prevalent APC variant that has been reported to date (3). The B1822V variant had an allele frequency of 22.8% in the predominately Caucasian control population of 1945 people. This compares to an allele frequency of 10% previously reported from a sample of 45 cases, race not reported (3) We observed that homozygous variant APC genotypes had a low BMI at lower risk of colon cancer than were those with a high BMI. However, those with a family history of colorectal cancer in a first-degree relative may have been at an elevated risk of colon cancer if they also carried the homozygous variant APC genotype.
It is involved in control of proliferation via β-catenin/Tcf and of mobility via interaction with the cytoskeleton. Although the functional significance of the 1822 substitution is uncertain at this time, the substitution changes the amino acid residue from a hydrophilic asparagine to a hydrophobic valine residue. Codon 1822 is located between the fourth and fifth of the seven 20-amino-acid repeats involved in the binding and down-regulation of β-catenin (22). Specifically, it is 180 amino acids downstream of the fourth repeat and 26 amino acids upstream of the fifth (1). Therefore, although this amino acid change is not conservative, there is no known consequence of an amino acid substitution at this location on β-catenin regulation or any other known function of APC. However, it is certainly possible that future functional and/or structural analyses of APC may suggest such consequences.

Modifier genes, genes that may modify the phenotypic manifestation of a mutated gene without necessarily having an effect on the wild type, have been identified for APC. One such gene, Mom1 (modifier of min; min is the mouse APC homologue), in the mouse (or the human homologue) encodes for the secretory type II phospholipase A2 (Pla2s) gene (23–25). Mom1 in the mouse modifies the phenotype associated with a mutated APC gene, resulting in fewer intestinal tumors (23). Pla2s is a phospholipase involved in generating arachidonic acid, which, in turn, is necessary for the synthesis of prostaglandins (24). These observations may lend some support to our finding that dietary fat interacts with this APC 1822 variant. If this particular gene-gene interaction is relevant to our findings, then it may be worth asking whether there is a protective phenotype associated with Mom1, even in the absence of a truncating polymorphism of APC.

There are several strengths of this study. Because of the large sample size, we have been able to assess interactions between diet and lifestyle factors in conjunction with a variant APC genotype. The dietary questionnaire used was comprehensive and, because of its link to the Nutrition Coordinating Center (NCC) nutrient database, has extensive information on dietary fat. On the other hand, we are limited by the fact that both cases and controls were asked to recall their diets from the past, and thus differential recall is possible. Finally, it is worth pointing out that the findings reported here may be the result of chance and have no biological significance. Therefore, need to be replicated in other populations with adequate sample sizes to evaluate the association.

Although associations between dietary fat and colon cancer are generally lacking at the population level, this study provides evidence of a small subset of the population who are at decreased risk of colon cancer as a result of consuming a low-fat diet. These findings, if replicated, may provide additional important clues to the etiology of colon cancer and to the functional role of the APC gene.

### Table 3: Associations between lifestyle factors and APC 1822 genotypes and risk of colon cancer

<table>
<thead>
<tr>
<th>APC genotypes</th>
<th>Wild-type</th>
<th>Heterozygote</th>
<th>Variant</th>
<th>Wild-type OR (95% CI)</th>
<th>Heterozygote OR (95% CI)</th>
<th>Variant OR (95% CI)</th>
<th>P interaction</th>
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<td>Physical activity</td>
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<td></td>
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<tr>
<td>Low</td>
<td>274/258</td>
<td>147/146</td>
<td>19/32</td>
<td>1.0</td>
<td>1.0 (0.7–1.3)</td>
<td>1.0 (0.7–1.3)</td>
<td>0.6 (0.3–1.1)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>410/488</td>
<td>213/281</td>
<td>39/34</td>
<td>0.8 (0.6–1.0)</td>
<td>0.7 (0.6–0.9)</td>
<td>0.8 (0.5–1.3)</td>
<td>0.4 (0.2–0.8)</td>
</tr>
<tr>
<td>High</td>
<td>288/450</td>
<td>183/220</td>
<td>17/35</td>
<td>0.6 (0.5–0.8)</td>
<td>0.8 (0.6–1.0)</td>
<td>0.4 (0.2–0.8)</td>
<td></td>
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<tr>
<td>BMI</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>High</td>
<td>422/421</td>
<td>220/218</td>
<td>34/37</td>
<td>1.0</td>
<td>1.0 (0.8–1.3)</td>
<td>0.9 (0.6–1.5)</td>
<td>0.43</td>
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<tr>
<td>Intermediate</td>
<td>291/387</td>
<td>163/220</td>
<td>18/36</td>
<td>0.8 (0.6–0.9)</td>
<td>0.7 (0.6–1.0)</td>
<td>0.5 (0.3–0.8)</td>
<td></td>
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<tr>
<td>Low</td>
<td>259/388</td>
<td>160/209</td>
<td>14/28</td>
<td>0.7 (0.6–0.8)</td>
<td>0.8 (0.6–1.0)</td>
<td>0.5 (0.3–1.0)</td>
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<td>Cigarette smoking (usual number of cigarettes/day)</td>
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<td></td>
<td></td>
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<tr>
<td>Never</td>
<td>385/560</td>
<td>229/314</td>
<td>31/47</td>
<td>1.0</td>
<td>1.1 (0.9–1.3)</td>
<td>0.9 (0.6–1.5)</td>
<td>0.52</td>
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<td>&lt;20</td>
<td>179/222</td>
<td>108/135</td>
<td>9/18</td>
<td>1.2 (1.0–1.5)</td>
<td>1.2 (0.9–1.6)</td>
<td>0.7 (0.3–1.5)</td>
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<td>≥20</td>
<td>404/408</td>
<td>198/196</td>
<td>26/35</td>
<td>1.4 (1.2–1.7)</td>
<td>1.4 (1.1–1.8)</td>
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<td>NSAIDs</td>
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<tr>
<td>No</td>
<td>597/625</td>
<td>337/333</td>
<td>41/51</td>
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<td>206/314</td>
<td>25/50</td>
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<td>0.5 (0.3–0.8)</td>
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<td>Family history of colorectal cancer</td>
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<tr>
<td>No</td>
<td>800/1085</td>
<td>459/584</td>
<td>55/89</td>
<td>1.0</td>
<td>1.1 (0.9–1.3)</td>
<td>0.8 (0.6–1.1)</td>
<td>0.70</td>
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<td>Yes</td>
<td>168/105</td>
<td>76/661</td>
<td>11/11</td>
<td>2.1 (1.6–2.8)</td>
<td>1.7 (1.2–2.4)</td>
<td>1.4 (0.6–3.4)</td>
<td></td>
</tr>
</tbody>
</table>

* Adjusted for age, sex, long-term physical activity patterns, NSAIDs, BMI, and dietary energy and fiber intake.
ACKNOWLEDGMENTS

We thank Sandra Edwards, Karen Curtin, KheNi Ma, and Melanie Nichols for the data collection and analyses and Drs. Bette Caan and Kristin Anderson for their contribution to data collection.

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