Ornithine Decarboxylase and Polyamines in Familial Adenomatous Polyposis


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Abstract

Familial adenomatous polyposis (FAP), due to germ-line mutation of the adenomatous polyposis coli (APC) gene, is characterized by development of colorectal adenomas and ultimately colorectal cancer. The usefulness of ornithine decarboxylase (ODC) activity and polyamine levels in normal-appearing colorectal mucosa to stratify risk for colorectal neoplasia by discriminating presymptomatic individuals with germ-line APC mutation (genotype-positive) from genotype-negative family controls was evaluated in 36 at-risk subjects undergoing endoscopic and genetic screening for FAP. ODC activity and levels of putrescine, spermidine, and spermine were significantly higher in presymptomatic genotype-positive individuals compared to genotype-negative persons (P = 0.029, <0.001, 0.002, and <0.001, respectively). Moreover, a putrescine level with a cutoff point of 1.5 nmol/mg protein was the most accurate single discriminator of risk status. ODC activity and polyamine levels are significantly elevated in gene carriers of FAP before the development of polyposis, suggesting a role for these compounds in tumorigenesis of FAP. These assays may be useful in evaluating at-risk members of FAP families in which mutation of the APC gene cannot be found.

Introduction

FAP is an autosomal dominant disease caused by germ-line mutation of the APC gene located on the long arm of chromosome 5 in band q21 (1). FAP is characterized by the development of hundreds of colorectal adenomas in young adults (2). If prophylactic colectomy is not performed, nearly all affected individuals will develop colorectal cancer by the sixth decade of life. Presymptomatic genetic testing for FAP is now available with the IVPS (3). IVPS testing of at-risk individuals can differentiate affected family members from those unaffected by FAP (3).

The polyamines (putrescine, spermidine, and spermine) are compounds required for cellular differentiation and proliferation (4). ODC is the first and rate-limiting enzyme in the polyamine biosynthetic pathway (4). Evidence suggests that ODC and polyamines have an important function in colorectal neoplasia. In murine models of colorectal carcinogenesis, carcinogens induce ODC activity in colonic mucosa, and inhibitors of ODC suppress cancer development (5–9). In human beings, ODC activity and polyamine levels appear increased in neoplasms compared to adjoining normal-appearing mucosa (10–18).

Several investigators have evaluated the use of ODC activity and polyamine levels in normal-appearing colorectal mucosa to stratify an individual's risk for colorectal neoplasia. Some groups have found a positive correlation between higher levels of these biomarkers and colorectal neoplasia (13, 16–22), whereas others have not found this association (23–27). Luk and Baylin (18) showed increased ODC activity in the colorectal mucosa of affected members of FAP families. In presymptomatic at-risk members with no evidence of adenomas, a biphasic distribution of mucosal ODC activity was noted, but these investigators had no way of knowing the genotypic status of the subjects. We, therefore, evaluated the ability of these biomarkers to discriminate the presence of risk of colorectal neoplasia in presymptomatic individuals with germ-line mutation of the APC gene from genotypically negative family controls.

Materials and Methods

Study Population. Thirty-six subjects recruited from 26 families in The Johns Hopkins Hereditary Colorectal Cancer Registry underwent screening for FAP because of an affected parent with known mutation of the APC gene identified by evaluation of peripheral blood leukocytes for mutation of the APC gene by IVPS (3). Informed consent was obtained from all subjects, and the protocol was approved by The Johns Hopkins University Joint Committee on Clinical Investigation (institutional review board). All subjects had normal flexible sigmoidoscopy, and biopsy specimens were histopathologically negative for adenomatous epithelial proliferation. Subjects then had presymptomatic genetic testing by IVPS.

The results of gene testing stratified subjects into two groups: affected patients with evidence of truncating mutation indicating future development of polyposis (designated genotype-positive), and unaffected persons with no mutation (designated genotype-negative).

Specimen Procurement. Mucosal ornithine decarboxylase activity and polyamine levels were measured in mucosal biopsy specimens taken by standard biopsy forceps during flexible sigmoidoscopy performed between 1 and 5 p.m. All subjects were prepared for the endoscopic procedure with clear liquid diet and oral cathartic solution. Enemas that could influence mucosal biochemistry were not given. In each patient, six rectal mucosal biopsy specimens were taken from flat mucosa 10–12 cm from the anal verge to minimize potential differences that might occur among specimens taken at different sites. Two biopsy specimens were snap frozen in liquid nitrogen for ODC analysis and two for polyamine analysis; two were placed in formalin for histopathological examination.

Laboratory Measurements. Ornithine decarboxylase activity in biopsy specimens was assayed according to the published methods of Seely and Pegg (28) as previously used in our laboratory (29). Enzyme determination was performed on replicates of cytosolic extracts. The polyamine contents of perchloric acid extracts of biopsy specimens were determined by reverse-phase, high-performance liquid chromatography with precolumn dansylation as described by Kabra et al. (30) as reported previously from our laboratory (29). 1,7-Diaminoheptane was used as the internal standard. This method has sensitivity to reproducibly detect >5 pmol of the individual polyamines and is reproducible with <15% variation. Proteins were quantitated by the methods of Bradford (31).

Statistical Analysis. Analysis for differences in demographic characteristics between genotype-positive and genotype-negative groups and mucosal ODC and polyamine levels was done by a two-tailed unpaired Student's t test; χ² test was used for categorical variables. A probability of P < 0.05 was considered statistically significant. Receiver operator characteristic curves were used to determine the accuracy of ODC activity and polyamine levels to
Table 1 Demographic characteristics of study subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Genotype positive (n = 25)</th>
<th>Genotype negative (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr): mean ± SE</td>
<td>14.3 ± 1.5 (range 8-44)</td>
<td>15.7 ± 1.1 (range 9-22)</td>
</tr>
<tr>
<td>Gender: no. (%)</td>
<td>Female 19 (76), Male 6 (24)</td>
<td>Female 6 (55), Male 5 (45)</td>
</tr>
<tr>
<td>Caucasian race: no. (%)</td>
<td>24 (96), 6 (24)</td>
<td>10 (90), 1 (9)</td>
</tr>
</tbody>
</table>

Table 2 Mean ODC activities and polyamine contents in genotype-positive and genotype-negative persons

<table>
<thead>
<tr>
<th>Parameter (mean ± SE)</th>
<th>Genotype positive (n = 25)</th>
<th>Genotype negative (n = 11)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ODC activity&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1592.57 ± 270.01</td>
<td>633.99 ± 146.59</td>
<td>0.029</td>
</tr>
<tr>
<td>Putrescine&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.625 ± 0.42</td>
<td>0.636 ± 0.082</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Spermidine</td>
<td>3.760 ± 0.174</td>
<td>2.880 ± 0.146</td>
<td>0.002</td>
</tr>
<tr>
<td>Spermine</td>
<td>9.374 ± 0.246</td>
<td>6.80 ± 0.33</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<sup>a</sup> ODC activities are expressed as pmol CO₂ per mg soluble protein per hour.
<sup>b</sup> Polyamine contents are given in nmol of polyamine per mg soluble protein.

Results

Thirty-six patients without endoscopic evidence of polyposis were separated by gene testing into two groups: 25 genotype-positive subjects and 11 genotype-negative subjects. There were no significant differences in demographic or clinical characteristics between the groups (Table 1). The mean ODC activity and levels of putrescine, spermidine, and spermine levels were significantly higher in genotype-positive patients compared to genotype-negative persons (Table 2; Fig. 1).

Analysis of the sensitivity and specificity of ODC activity and polyamine levels by receiver operator characteristic curves (Fig. 2) revealed that putrescine levels best discriminated between genotype-positive and genotype-negative individuals. A putrescine cutoff level of 1.5 nmol/mg protein was 96% sensitive and 100% specific for the diagnosis of FAP; the predictive value of a positive and negative test was 100 and 92%, respectively.

Discussion

In our study, patients with mutation of the APC gene but no colorectal polyposis had significantly increased ODC activity and polyamine levels compared to unaffected family members. Similarly, in murine studies of colorectal carcinogenesis, tumor-producing carcinogens induce ODC mucosal activity in normal-appearing colonic mucosa of treated animals destined to have tumors (5—7).

Our data corroborate literature reports describing discrimination of colorectal neoplasia risk in humans by assay of compounds in the polyamine pathway (13, 16—22). Our results in presymptomatic at-risk subjects who were categorized by genotype also provide an explanation for the previously observed biphasic distribution of ODC activities and polyamine levels over a range of cutoff points.

Fig. 1. Scatter plots showing biomarker values for genotype-positive and genotype-negative individuals for ornithine decarboxylase activity (A), putrescine level (B), spermidine level (C), and spermine level (D).
activity noted in at-risk relatives of FAP patients in the study by Luk and Baylin (18); at-risk persons with elevated mucosal ODC activity were probably genotypically affected. Yet, some authors have not found ODC activity and polyamine levels to differentiate risk (23–27). Many reports concern patients with sporadic adenomas and colorectal cancer rather than hereditary forms; different and/or subtler mechanisms of carcinogenesis are likely to be important. Interestingly, Love et al. (26) reported that ODC activity did not discriminate members in hereditary nonpolyposis colorectal cancer families, another inherited colorectal cancer syndrome due to germ-line mutation of one of a family of genes responsible for repair of DNA nucleotide mistakes. Application of gene status to such studies would be useful.

The ability of ODC and polyamines to diagnose FAP was analyzed by receiver operator characteristic curves. Putrescine levels had the best accuracy at a cutoff level of 1.5 nmol polyamine/mg soluble protein. By contrast, ODC activity and other polyamines had substantial overlap, which limits clinical use. Mucosal putrescine level may be important because, with present technology, an APC gene mutation cannot be identified in about 20% of FAP families (3). Consequently, presymptomatic genetic testing is not available; therefore, endoscopic screening is mandatory in all such at-risk persons. Studies of ODC activity and polyamine levels in FAP families without identifiable APC mutation are needed to determine if these markers can be used in risk assessment.

Acknowledgments

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References

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