Heredity nonpolyposis colorectal cancer
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Proximal adenomas in hereditary nonpolyposis colorectal cancer are prone to rapid malignant transformation

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Abstract

**Background** - Hereditary nonpolyposis colorectal cancer (HNPCC) is thought to arise from adenomas. HNPCC mostly occurs in the proximal colon. We investigated whether this proximal preponderance is due to a proximal preponderance of adenomas or (also) due to differences in transformation rates from adenomas to cancer between distal and proximal colon.

**Methods** - 100 HNPCC adenomas were evaluated and compared to 152 sporadic adenomas for location, size and dysplasia. 25 adenomas from patients with a known mismatch repair gene (MMR) mutation were stained for expression of *MLH1* and *MSH2*.

**Results** - HNPCC adenomas were more often located proximally (50% vs. 26%, p=0.018) and were smaller in comparison to sporadic adenomas. They were similarly dysplastic. However, all proximal HNPCC adenomas ≥5 mm were highly dysplastic compared to 17% of the larger proximal sporadic polyps (p<0.001). They were also more often highly dysplastic than larger distal HNPCC adenomas (p<0.001). Small HNPCC adenomas were, except for their location, not different from sporadic ones. 15 of the 25 'known-mutation' adenomas showed loss of expression of either *MLH1* or *MSH2*. The 10 adenomas with expression were all small and low-grade dysplastic.

**Conclusion** - HNPCC adenomas are located mainly in the proximal colon. The development to high-grade dysplasia is more common in proximal HNPCC adenomas than in distal ones, indicating a faster transformation rate from early adenoma to cancer in the proximal colon. MMR-gene malfunction probably does not initiate adenoma development but is present at a very early stage of tumorigenesis and heralds the development of high-grade dysplasia.
For the first time, researchers have obtained indisputable data supporting the usage of contemporary colorectal cancer prevention procedures in hereditary nonpolyposis colorectal cancer (HNPCC) syndrome.\textsuperscript{1} These data reveal a significant patient survival advantage and an incidence reduction of colorectal tumors due to colonoscopic screening and polypectomies. This complies with the accepted adenoma-carcinoma sequence theory that states that adenomas are a precursor in the tumorigenesis of malignant HNPCC lesions.\textsuperscript{2-4}

The malignant lesions, often occurring at relatively young age, are well described in HNPCC. They are located predominantly in the proximal part of the colon, and there is a high incidence of synchronous and metachronous cases. Microscopically the tumors are characterized by a Crohn’s like lymphoid reaction, a mucinous component and by poor differentiation.\textsuperscript{5-10}

Publications on the adenomas in HNPCC are less consistent. When calculating the average distribution of HNPCC adenomas mentioned in the literature, forty-five percent (range: 27-70%) is located in the proximal colon.\textsuperscript{1,9,11-14} Some reported an obvious propensity for right-sided neoplastic lesions while others observed a distribution of adenomas in HNPCC patients similar to that in the general population.\textsuperscript{13,15-17}

The adenoma-carcinoma sequence in HNPCC seems to be accelerated. This is especially illustrated by the relatively frequent occurrence of cancers within the first few years after a "clean" colon had been confirmed by colonoscopy.\textsuperscript{14-15,18-19} In addition, several authors reported that HNPCC adenomas frequently have a villous component and high-grade dysplasia, two assumed markers of increased risk to develop cancer.\textsuperscript{9,11,14,20} However, whether this is a uniform feature for all HNPCC adenomas at every location in the colon has yet to be determined. The ratio of proximal to distal cancers in HNPCC (7:3) is higher than the reported ratio for adenomas (4:5). It thus seems that not all HNPCC adenomas have an increased risk for malignant transformation and that there are regional differences in this respect.

In order to examine the above-mentioned issues, we compared the adenomas resected from HNPCC patients to sporadic adenomas. More importantly, we investigated whether differences exist between proximal, distal and rectal HNPCC adenomas.
Methods

Patient characteristics

According to the prevention guidelines of the International Collaborative Group on HNPCC (ICG-HNPCC) people fulfilling the Amsterdam criteria and/or having a germline mismatch repair gene mutation should undergo a colonoscopy every two years starting at the age of 25.\textsuperscript{21-23} At the University Hospital of Groningen 136 persons, belonging to 47 families, participate in a surveillance program. We included all subjects with a positive colonoscopy in our study group: 46 (24 male and 22 female) patients with a median age of 50 (range 25-78) years at polypectomy. Sixty-nine colonoscopies, a mean of 1.6 per person (range 1-5), yielded 100 adenomatous polyps (55 from men and 45 from women) in a 12-year period, from 1988 to 2000. Four polyps found in a subtotal colectomy specimen were also included. Seven persons had previously been diagnosed with cancer and had part of their colon resected.

The data were compared to the findings from a control group consisting of the sporadic adenomas consecutively removed during sigmoido- and colonoscopy at the Endoscopy Center of the University Hospital of Groningen in 1997. Lesions from patients with a strong positive family history of colorectal cancer, or patients having ulcerative colitis, Crohn’s disease or familial adenomatous polyposis (FAP) were not included. According to protocol patients with an adenoma detected at sigmoidoscopy should subsequently have a colonoscopy: except for two patients, the entire large bowel was inspected in each person.\textsuperscript{24} The group of sporadic adenomas consisted of 152 adenomas and had a similar male-female ratio as in the HNPCC group, 84 lesions from men and 68 from women. The average age at polypectomy was 64 (range 24-90) years.

Location

The location of the adenomas was retrieved from endoscopy or pathology reports. The caecum and the ascending and transverse colon are regarded as the proximal or right-sided colon while the descending and the sigmoid colon are referred to as the distal or the left-sided colon. The third location of the adenomas was the rectum.
**Histological examination**

Thin slides, 3µm, were made of each formalin-fixed paraffin-embedded polyp and stained with hematoxylin-eosin (HE). Two authors, Rijcken and Hollema, reviewed and scored three characteristics of the adenomas: 1. Size of the polyp - the microscopic measurement of the polyp’s circumference, < 5mm (small) and ≥ 5mm (large); 2. Histological subtype- tubular or having more than a 25% villous component; 3. Grade of dysplasia- low or high (WHO guidelines).

The adenomas, 25 in total, which were removed from persons with a known mutation or belonging to a family with a known mutation were further analyzed for mismatch repair (MMR) protein status by immunohistochemistry. Monoclonal mouse antibodies against *MLH1* (clone G168-728, Pharmingen, San Diego, USA) and *MSH2* (Ab-2, Calbiochem, San Diego, USA) protein products were used. The paraffin sections (3µm) were fixed onto 3-aminopropyltriethoxysilane (APES, Sigma-Aldrich, Diesenhofen, Germany) coated slides, stretched for 30 minutes at 60°C and dried overnight at 37°C. The sections were deparaffinized in xylene (2x 10 minutes) and rinsed in 100% alcohol. The optimal antibody-antigen reaction was obtained by immersing the section in 200µl blocking reagent (2% block and 0.2% SDS in maleic acid, pH 6.0 (Boehringer Mannheim, Germany)) and using a high pressure cooker for 3 sessions of 5 minutes at 115°C alternating with 5 minutes of incubation in a humid environment. After cooling for the third time, endogenous peroxidase activity was quenched by incubation with 30% H₂O₂ in phosphate buffered sulfate (PBS) for 30 minutes. Following thorough washing in PBS, the sections were immersed with the specific antibody in PBS with 1% bovine serum albumin (BSA), at a dilution of 1:500 for *MLH1* and 1:100 for *MSH2* antibody, for one hour. Subsequently, the sections were washed three times with PBS and consecutively incubated for 30 minutes with rabbit antimouse peroxidase and goat antirabbit peroxidase diluted (1:50) in PBS-1% BSA. The sections were submerged for 10 minutes in a solution of 50 mg 3’-3’diaminobenzidine in PBS and 50 mg of imidazol with 30% H₂O₂, used as substrate of peroxidase. After rinsing with demi water, the sections were counterstained with haematoxylin, washed with running water and dehydrated with graded alcohol, dried and covered with a slide.
**Statistical analyses**

Statistical comparisons between HNPCC and sporadic adenomas and within the two groups were made using the Mann-Whitney test when comparing a characteristic (size, type and dysplasia) with two variables and the corrected Chi-squared test was used in the comparisons of a characteristic (location) with three variables. A p-value of less than 0.05 was considered to be statistically significant.

**Results**

Taking the two entire groups of adenomas in consideration (table 1), a significant difference can be observed between the location, size and histology of HNPCC and that of sporadic adenomas (p= 0.018, p< 0.001 and p= 0.010, resp.). HNPCC adenomas had a proximal propensity (50% vs 26% of the sporadic adenomas). The adenomas showed a wide range of sizes in both groups. The median size of HNPCC adenomas was 2.0 mm (range 0.5-34mm), while the sporadic adenomas had a medium size of 5.5mm (range 0.5- 45mm). The HNPCC adenomas were more often tubular in comparison to the sporadic adenomas. Even though the HNPCC adenomas were more often small and tubular they were similarly dysplastic as the larger and more often villous sporadic adenomas.

**Table 1. The four characteristics of HNPCC and sporadic adenomas by number and percentage**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HNPCC n (%)</th>
<th>Sporadic n (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Location</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal</td>
<td>47 (50)</td>
<td>39 (26)</td>
<td>0.018</td>
</tr>
<tr>
<td>Distal</td>
<td>29 (30)</td>
<td>72 (47)</td>
<td></td>
</tr>
<tr>
<td>Rectum</td>
<td>19 (20)</td>
<td>41 (27)</td>
<td></td>
</tr>
<tr>
<td><strong>Size</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 5 mm</td>
<td>70 (70)</td>
<td>67 (44)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>≥ 5 mm</td>
<td>30 (30)</td>
<td>85 (56)</td>
<td></td>
</tr>
<tr>
<td><strong>Type</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tubular</td>
<td>79 (79)</td>
<td>97 (64)</td>
<td>0.010</td>
</tr>
<tr>
<td>Tubulovillous</td>
<td>21 (21)</td>
<td>55 (36)</td>
<td></td>
</tr>
<tr>
<td><strong>Dysplasia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-grade</td>
<td>68 (68)</td>
<td>114 (76)</td>
<td>0.567</td>
</tr>
<tr>
<td>High-grade</td>
<td>32 (32)</td>
<td>38 (25)</td>
<td></td>
</tr>
</tbody>
</table>
The proximal propensity was more evident in highly dysplastic HNPCC adenomas: high-grade dysplastic HNPCC adenomas were more often (55%) located proximal to the splenic flexure while only 6 (15%) of the high-grade sporadic polyps were right-sided (p< 0.001).

In the proximal colon the HNPCC adenomas were smaller in comparison to sporadic polyps (p=0.025) but they were more often highly dysplastic (36% vs 13%, p<0.020) (figure 1). When comparing size as well as dysplasia at one location (table 2A&B), the difference between the HNPCC and sporadic adenomas was clearly apparent. Especially larger HNPCC polyps, ≥ 5mm, in the proximal colon were more often highly dysplastic (p<0.001): in fact, all HNPCC adenomas equal to or larger than 5 mm were highly dysplastic. In the sporadic lesions this was not observed: only 25% of the large sporadic polyps at this site were high-grade dysplastic.

Table 2A. Dysplasia of HNPCC adenomas by location and size

<table>
<thead>
<tr>
<th></th>
<th>Proximal</th>
<th>Distal</th>
<th>Rectum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dysplasia</td>
<td>low</td>
<td>high</td>
<td>low</td>
</tr>
<tr>
<td>&lt; 5 mm</td>
<td>30</td>
<td>17</td>
<td>25</td>
</tr>
<tr>
<td>≥ 5 mm</td>
<td>0</td>
<td>11</td>
<td>8</td>
</tr>
</tbody>
</table>

* Difference between ≥ 5mm adenomas in the proximal and distal colon and between proximal colon and rectum: p value=0.009 and p=0.07, respectively.

Table 2B. Dysplasia of sporadic adenomas by location and size

<table>
<thead>
<tr>
<th></th>
<th>Proximal</th>
<th>Distal</th>
<th>Rectum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dysplasia</td>
<td>low</td>
<td>High</td>
<td>low</td>
</tr>
<tr>
<td>&lt; 5 mm</td>
<td>34</td>
<td>5</td>
<td>45</td>
</tr>
<tr>
<td>≥ 5 mm</td>
<td>15</td>
<td>3</td>
<td>23</td>
</tr>
</tbody>
</table>

* Difference between ≥ 5mm adenomas in the proximal and distal colon and between proximal colon and rectum: Both p values are not significant.
In the rectum, large HNPCC adenomas were also more often highly dysplastic in comparison to sporadic adenomas ≥ 5 mm. However, this difference was only borderline significant (p=0.084). The HNPCC adenomas in the distal colon were smaller than distal sporadic lesions (66% vs 39% <5mm, p=0.047), mostly tubular (72% vs 59%, not significant) and nearly always low-grade dysplastic (86% vs 63%, p=0.020).

A significant difference was not only observed between the HNPCC and sporadic groups but also between the large HNPCC adenomas at the three locations. The large proximal HNPCC adenomas were more often highly dysplastic (100% were highly dysplastic) than the HNPCC adenomas in the distal colon (22%, p=0.001), whereas a borderline significant difference was detected between proximal and rectal adenomas (75% highly dysplastic, p=0.07). The large HNPCC adenomas in the rectum were also significantly more often highly dysplastic in comparison to the HNPCC adenomas in the distal colon (p<0.03).
Eleven adenomas belonged to patients with a proven *MLH1* mutation while 11 adenomas were removed from persons with a proven *MSH2* mutation. Three adenomas were from two persons who had not undergone genetic testing but in their families an *MLH1* mutation had been detected. In total, 15 adenomas, 8 low-grade and 7 high-grade dysplastic, showed loss of expression of either MLH1 or MSH2. The three adenomas from the two persons who had not undergone genetic testing all showed loss of MLH1 staining, corresponding to the known mutation in their kindreds. Eight adenomas with loss of MLH1 expression were located in the proximal colon and 2 were situated in the rectum. Loss of MSH2 expression was observed in 3 distal, 1 proximal and 1 rectal polyp. The 10 adenomas, all from proven carriers, that had normal expression of MMR proteins were small and low-grade dysplastic. Three of these adenomas were located in the proximal colon, four in the distal colon and the remaining three were located in the rectum.

**Discussion**

Our results differ, as will be explained below, from previous studies concerning the morphological descriptions of adenomas in persons at risk for hereditary nonpolyposis colorectal cancer. The results support the accelerated adenoma-carcinoma sequence theory of carcinogenesis in HNPCC but at the same time strongly suggest that lesions at different locations, proximal or distal in the colon or in the rectum, behave differently in this respect. More specifically, similarly to the high frequency of hereditary nonpolyposis colorectal carcinomas proximal to the splenic flexure, HNPCC adenomas have a right preponderance, though less so than the carcinomas, and these right-sided adenomas are more prone to malignant conversion in comparison to the left-sided adenomas.

The large group of HNPCC adenomas included in this study is representative for the Groningen HNPCC population, as they are all the available benign neoplastic lesions consecutively removed during a twelve year period. The majority of the HNPCC adenomas were obtained during surveillance examinations, while the sporadic adenomas were acquired during a colonoscopy to diagnose clinical symptoms such as rectal blood loss, diarrhea and abdominal pain. The groups are thus not readily comparable and this probably explains why the HNPCC adenomas were smaller, more often tubular and were not more dysplastic than the sporadic ones, findings that are in contrast with what has been reported in the
It is noticeable, however, that the small and tubular HNPCC adenomas were not less dysplastic than the larger and more often villous sporadic cases, as would be expected. To reduce the discrepancy arisen by comparing adenomas obtained through surveillance and diagnostic colonoscopies we also evaluated only the index HNPCC adenomas, those adenomas found during the first colonoscopy (data not shown). The characteristics of these index neoplastic lesions did not differ from the characteristics of all the HNPCC adenomas reported in the present paper. In accordance to Green's observation, they were mostly small and tubular. The same team of gastroenterologists did all colonoscopies. The number of small, <5mm, HNPCC and sporadic adenomas in all sections of the colon illustrates the thorough endoscopic search in both groups and through the entire colon. Nevertheless, it can not be excluded that even greater care was taken in the examination of the colons from HNPCC subjects than of those from other patients.

The prevalence and grade of dysplasia of adenomas in the general population differ per gender, age and geographic region. Any bias was minimized by including the same male to female ratio of adenomas in both groups. A sufficient amount of age matched subjects, however, was not available. The histological characteristics of our control group are similar to the results of Griffioen et al, also a Dutch clinical investigation. The results from the control group also correspond to findings of several other publications. However, yet other reports, mainly autopsy and surveillance studies, favor a substantially higher frequency of low-grade dysplastic sporadic polyps and a lower frequency of villous lesions. The proportion of proximal adenomas varies in the literature between 11% and 40% (Griffioen et al, 17%).

The essential role of the proximal colon in the pathogenesis of HNPCC is evident from numerous reports of the high incidence of right-sided (interval) carcinomas in HNPCC patients. In accordance with this we found that HNPCC adenomas are located more often in the proximal colon than sporadic ones. The proximal propensity suggests an alteration in initiation of neoplastic growth in HNPCC in comparison to the general population. A recent report on the presence of microsatellite instability in both hyperplastic and dysplastic aberrant crypt foci in colons from HNPCC-patients also suggests a possible role of MMR dysfunction in the initiation of neoplastic lesions in HNPCC. However, Leach
among others, proposed that the adenomas in HNPCC develop on a sporadic basis only to provide a substrate for defective DNA mismatch repair genes.\textsuperscript{44}

We therefore tried to substantiate either the Leach theory or the 'initiation' proposal by performing immunohistochemistry (IHC) for gene products of \textit{MLH1} and \textit{MSH2} on the adenomas from persons with a known mutation. In accordance with most reports, the absence of immunohistochemical staining was seen in two-thirds of the HNPCC adenomas, i.e. in 44% of the low-grade and in all high grade dysplastic adenomas.\textsuperscript{45-50} Thus, our data strongly suggest that DNA repair deficiency is not responsible for the initiation of an adenoma but determines the subsequent progression of the lesion. Although MMR dysfunction is not the first event, it is surely a very early one in the tumorigenesis of HNPCC lesions and it heralds development to high-grade dysplasia.

Our results illustrate that the proposed accelerated adenoma-carcinoma sequence in HNPCC is probably site-specific i.e. limited to the proximal colon. While the group of HNPCC adenomas as a whole did not exhibit features of increased susceptibility to malignant conversion in comparison to sporadic adenomas, a difference was observed within the group of adenomas proximal to the splenic flexure. The large (≥5mm) HNPCC adenomas in the proximal bowel were all highly dysplastic suggesting more advancement in the malignant transformation than sporadic polyps of similar size at this location. This and the fact that 9 of the 15 HNPCC adenomas that showed loss of protein expression were located in the proximal colon emphasize the predilection of the proximal colon for tumorigenesis in HNPCC. Characteristics of HNPCC adenomas at specific sites in the large bowel have had limited attention so far as authors have reported results on HNPCC adenomas only in general. On the other hand sporadic adenomas have been described per location. The National Polyp Study observed an increased frequency of high-grade dysplasia in adenomas located distal to the splenic flexure, but attributed it mainly to increased size and villous component rather than to location per se.\textsuperscript{27} Nusko and colleagues reported that sporadic right-sided adenomas have a lower risk to become malignant in comparison to sporadic left-sided adenomas.\textsuperscript{51} The reasons for the reverse situation in HNPCC are still unclear.\textsuperscript{25,52-54}

In conclusion, half of the HNPCC adenomas are located in the proximal colon, but this does not fully explain the proximal propensity of cancers. Our data show that development to high-
grade dysplasia is more common in proximal HNPCC adenomas than in distal ones, indicating a faster transformation rate from early adenoma to cancer in the proximal colon. MMR-gene malfunction probably does not initiate adenoma development but is present at a very early stage of tumorigenesis and seems to herald the development of high-grade dysplasia. At this time there is no ready explanation for this site-specific behavior of HNPCC-lesions or for the susceptibility of the epithelial cells in the proximal colon for somatic MMR-gene mutations.
References


