The serrated neoplasia pathway: investigating the role of serrated polyps in colorectal cancer development
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Hyperplastic polyps and sessile serrated adenomas as phenotypic expression of MYH-associated polyposis (MAP)

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ABSTRACT

**Background and aims:** MYH-associated polyposis (MAP) is a disorder caused by a bi-allelic germline MYH mutation, characterised by multiple colorectal adenomas. These adenomas typically harbour G:C→T:A transversions in the *APC* and *K-ras* genes caused by MYH deficiency. Occasional hyperplastic polyps (HPs) have been described in MAP patients but a causal relationship has never been investigated.

We examined the presence of HPs and sessile serrated adenomas (SSAs) in 17 MAP patients and studied the occurrence of G:C→T:A transversions in the *APC* and *K-ras* gene in these polyps. **Methods:** MAP patients were analysed for the presence of HPs/SSAs. *APC*-mutation cluster region and *K-ras* codon 12 mutation analysis was performed in adenomas (n=22), HPs (n=63) and SSAs (n=10) from these patients and from a control group of sporadic adenomas (n=17), HPs (n=24) and SSAs (n=17). **Results:** HPs/SSAs were detected in 8/17 (47%) of MAP patients of which 3 (18%) met the criteria for hyperplastic polyposis syndrome (HPS). *APC* mutations were only detected in adenomas and comprised exclusively G:C→T:A transversions. *K-ras* mutations were detected in 51/73 (70%) HPs/SSAs in MAP patients, compared to 7/41 (17%) in sporadic HPs/SSAs in the control group (p<0.0001). In HPs/SSAs, 48/51 (94%) of *K-ras* mutations exhibited G:C→T:A transversions, compared to 2/7 (29%) of sporadic HPs/SSAs in the control group (p<0.0001).

**Conclusions:** HPs and SSAs are a common finding in MAP patients. The detection of almost exclusively G:C→T:A transversions in the *K-ras* gene of HPs/SSAs strongly suggests that these polyps are causally related to MYH deficiency. This implies that distinct pathways, i.e. *APC*-gene related in adenomas and non-related in HPS/SSAs, appear to be operational in MAP.


**Background and aims**

Familial adenomatous polyposis (FAP) is an autosomal dominant disorder characterised by the development of hundreds of colorectal adenomas and eventually colorectal cancer (CRC) at young age. FAP is caused by a germline mutation in the *APC* tumor suppressor gene.

A milder form of FAP, known as attenuated FAP (AFAP), is caused by mutations in the extreme distal or proximal portion of the *APC* gene resulting in fewer adenomas and an older age of onset.\(^1,2\)

Recently, an autosomal recessive polyposis syndrome caused by bi-allelic germline mutations in the mutY human homologue (*MYH*) gene has been identified, known as *MYH* associated polyposis (MAP). *MYH* is a DNA glycosylase that plays a key role in base excision repair (BER) of 8-oxoG:A mismatches caused by reactive oxygen species.\(^3\)

Deficiency of this pathway results in somatic G:C→T:A transversions that are indeed found in the *APC* and K-ras genes in adenomas of these patients.\(^4-6\) Clinically, MAP resembles AFAP with an average age of onset around the mid 50’s and often fewer than 100 adenomas, predominantly in the proximal colon. Polyps have been reported to be mainly tubular adenomas, some tubulo-villous adenomas and occasional hyperplastic polyps (HPs).\(^6-9\)

Chow et al reported one patient with a bi-allelic *MYH* mutation and multiple HPs in addition to adenomatous polyposis.\(^10\) Here we describe 17 MAP patients of which 8 (47%) harboured HPs and sessile serrated adenomas (SSAs) in addition to conventional adenomas. We provide evidence that the HPs and SSAs in MAP patients are not rare and are causally associated with the *MYH* deficiency, reflected by G:C→T:A transversions in K-ras mutated genes of these polyps.


Materials and Methods

Patients and specimens

For this study, our cohort of 17 patients with a bi-allelic MYH mutation, receiving treatment at the endoscopy department of the Academic Medical Center (Amsterdam) from 21-7-1988 to 12-3-2008, was analysed for the presence of HPs or SSAs. MAP patients with HPs/SSAs were arbitrarily classified into two groups: patients with multiple HPs and SSAs (≥10); or patients with occasional HPs and SSAs (<10).

Polyp characteristics were recorded retrospectively from previous colonoscopy reports or the gross description of the resection specimens. All polyps were blindly re-evaluated and diagnosed separately by two experienced pathologists as HP, SSA or adenoma. SSA was defined by irregular crypts with architectural distortion, serration and dilation extending to the base of the crypts that often display boot- or T-shaped branching, and abnormal proliferation and maturation with mature goblet or foveolar cells at the base of the crypts.¹¹ In case of disagreement, consensus was reached during a multi-headed microscope session. This study was conducted in accordance with the research code of our institutional medical ethical committee on human experimentation, as well as in agreement with the Helsinki Declaration of 1975, as revised in 1983.

Somatic mutation analysis

Epithelial cells from HPs (n=63), SSAs (n=10) and adenomas (n=22) were microdissected and DNA was isolated as described previously.¹² In addition, DNA was isolated from sporadic HPs (n=24) and SSAs (n=17) of similar average size (median 3mm, range: 2-12mm), from a randomly selected group of patients without a polyposis syndrome.
(control group). Using previously described primers and assays, DNA was analysed for mutations in the APC-mutation cluster region (codon 1250-1550), K-ras codon 12. Detected mutations were confirmed in a second experiment.

**Statistics**

Statistical analyses were performed by using a statistical software package (Statistical Package for the Social Sciences 12.0.2; SPSS Inc, Chicago, Ill). Somatic K-ras codon 12 and APC-mutation cluster region (APC-MCR) configurations were compared with those of a control panel using a two-sided Fisher exact test. A p-value of < 0.05 was considered statistically significant.

**Results**

From 17 patients with MAP, 8 (47%) unrelated patients were identified having at least 1 HP and/or SSA. Of these 8 patients, 3 had >10 HPs and/or SSAs (table 1). The median age was 50 (range 34-67) years. Besides multiple adenomas (median 25, range: 3-39), a total of 145 HPs and 19 SSAs were identified from biopsies and polypectomy specimens (figure 1). The median size of detected HPs and SSAs was 3mm (range: 2-9 mm).
<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Resection</th>
<th>HPs(^1) analyzed/total</th>
<th>SSAs(^1) analyzed/total</th>
<th>Median size HPs/SSAs (range mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>67</td>
<td>F</td>
<td>TC</td>
<td>24/106</td>
<td>8/17</td>
<td>4(2-6)/4(2-6)</td>
</tr>
<tr>
<td>2</td>
<td>46</td>
<td>M</td>
<td>None</td>
<td>13/13</td>
<td>1/1</td>
<td>2 (2-3)/3</td>
</tr>
<tr>
<td>3</td>
<td>67</td>
<td>F</td>
<td>RC</td>
<td>14/14</td>
<td>0/0</td>
<td>2(2-3)</td>
</tr>
</tbody>
</table>

MAP patients with occasional (<10) hyperplastic polyps/sessile serrated adenomas

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Resection</th>
<th>HPs(^1) analyzed/total</th>
<th>SSAs(^1) analyzed/total</th>
<th>Median size HPs/SSAs (range mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>50</td>
<td>M</td>
<td>None</td>
<td>2/2</td>
<td>1/1</td>
<td>4(2-5)/4</td>
</tr>
<tr>
<td>5</td>
<td>49</td>
<td>F</td>
<td>STC</td>
<td>3/3</td>
<td>0/0</td>
<td>2(2-8)</td>
</tr>
<tr>
<td>6</td>
<td>47</td>
<td>M</td>
<td>STC</td>
<td>4/4</td>
<td>0/0</td>
<td>5(2-9)</td>
</tr>
<tr>
<td>7</td>
<td>79</td>
<td>F</td>
<td>STC</td>
<td>1/1</td>
<td>0/0</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>34</td>
<td>F</td>
<td>TC</td>
<td>2/2</td>
<td>0/0</td>
<td>2</td>
</tr>
</tbody>
</table>

Total 50 | 63/145 | 10/19 | 3 (2-9) |

Table 1. Histologically confirmed hyperplastic polyps and sessile serrated adenomas in 8 patients with a bi-allelic MYH mutation. HP: hyperplastic polyp; SSA: sessile serrated adenoma; TC: total colectomy; STC: subtotal colectomy; RC: right colectomy

\(^1\)Biopsied polyps

A (hemi)colectomy was performed in 6/8 patients, due to concerns that their polyps were too numerous to guarantee adequate colonoscopic surveillance, resulting in the detection of most polyps in the remaining distal colon or rectum. In all 3 patients with multiple HPs and/or SSAs, more polyps (>30) were seen than biopsied (figure 2).
Somatic APC and K-ras mutations

From the patients with MAP, 22 adenomas, 63 HPs and 10 SSAs were analysed for APC-MCR and K-ras codon 12 mutations. In adenomas, APC mutations were found in 9/22 (41%) of polyps, and comprised exclusively G:C $\rightarrow$ T:A transversions, whereas no G:C $\rightarrow$ T:A transversions were found in APC mutated adenomas in the control group (p<0.0001). K-ras mutations were found in 5/22 (23%) of adenomas, also comprising exclusively G:C $\rightarrow$ T:A transversions.

In HPs and SSAs of MAP patients and of the control group, no APC mutations were detected (table 2). K-ras mutations were detected in 51/73 (70%) of HPs/SSAs in MAP patients, which was significantly more than the frequency in sporadic HPs/SSAs (7/41: 17%) in the control group (p<0.0001). Furthermore, in K-ras mutated HPs/SSAs from our patients with MAP 48/51(94%) of mutations comprised a G:C $\rightarrow$ T:A transversion (figure 3), compared to 2/7 (29%) of mutations in sporadic HPs/SSAs in the control group (p<0.0001).
Figure 1. Examples of the spectrum of lesions found in the colon: hyperplastic polyp (top panel), sessile serrated adenoma (middle panel) and tubular adenoma (bottom panel).
Association between serrated polyps and MYH-associated polyposis

Table 2. Detected APC-mutation cluster region and K-ras codon 12 mutations in hyperplastic polyps (HP), sessile serrated adenomas (SSA) and adenomas (AD) and distribution of G:C→T:A transversions compared to a control panel.

<table>
<thead>
<tr>
<th>Polyps</th>
<th>Patients with MYH-associated polyposis (n=8)</th>
<th>Control group</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AD (n=22)  HP (n=63)  SSA (n=10)</td>
<td>AD (n=17)  HP (n=24)  SSA (n=17)</td>
<td></td>
</tr>
<tr>
<td>APC mutation</td>
<td>9 0 0</td>
<td>7 0 0</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>G:C→T:A</td>
<td>9(100%) 0 0</td>
<td>0 0 0</td>
<td></td>
</tr>
<tr>
<td>K-ras mutation</td>
<td>5 45 6</td>
<td>4 5 2</td>
<td>&lt;0.0001**</td>
</tr>
<tr>
<td>G:C→T:A</td>
<td>5(100%) 42 (93%) 6 (100%)</td>
<td>3 (75%) 1 (20%) 1 (50%)</td>
<td>&lt;0.0001**</td>
</tr>
</tbody>
</table>

Table 2. Detected APC-mutation cluster region and K-ras codon 12 mutations in hyperplastic polyps (HP), sessile serrated adenomas (SSA) and adenomas (AD) and distribution of G:C→T:A transversions compared to a control panel.

*Statistically significant p-value for adenomas in patients with MYH-associated polyposis (MAP) compared to adenomas in the control group

**Statistically significant p-value for HPs/SSAs in patients with MAP compared to HPs/SSAs in the control group.

Discussion

MAP is an autosomal recessive polyposis syndrome, caused by a bi-allelic germline MYH gene mutation. Similar to FAP, MAP is characterised by the presence of multiple adenomas in the colorectum and a high cancer risk. Here, we show that HPs and SSAs can also be considered a phenotypic expression of MAP, reflected by the detection of HPs/SSAs in 8/17 (47%) of patients of which 3 (18%) also met the criteria for hyperplastic polyposis syndrome (HPS). Interestingly, in previous large series of patients with MAP, only ‘occasional’ HPs and no SSAs were described. The presence of HPs/SSAs in combination with adenomas may thus be more common than previously thought. A possible cause for this discrepancy may lie...
in the fact that HPs/SSAs are more difficult to detect endoscopically due to their often diminutive size and flat or sessile shape. Furthermore, in patients with multiple adenomatous polyps like in MAP, the finding of small hyperplastic-looking polyps at colonoscopy may seem of little clinical interest and are consequentially not biopsied, preventing documentation of HPs/SSAs as a phenotypic feature of MAP.

Figure 2. Endoscopic picture of multiple hyperplastic polyps in a patient with MYH-associated polyposis.

HPS is a recently recognised condition, frequently linked with CRC and defined as at least five HPs proximal to the sigmoid colon, of which two are greater than 10mm in diameter, or more than 20 HPs distributed throughout the colon.\textsuperscript{15-18} Besides multiple HPs, the presence of SSAs, traditional serrated adenomas and conventional adenomas are common findings in this condition.\textsuperscript{10, 19} The combination
of *BRAF* mutations and CPG-island methylation (CIMP), evolving in SSAs, are considered to be the key mechanism in the ‘serrated neoplasia pathway’ leading to CRC in these patients.\textsuperscript{20-23} In our cohort of MAP patients, 3 unrelated patients also met the criteria for HPS. Two of these bi-allelic *MYH* mutations carriers were compound heterozygote (Y176C and P402L; Y165C and G382D) and one was monozygote (G382D). Most HPs/SSAs from these patients were detected in the distal colon and rectum and not proximally. This could partly be due to the fact that 2/3 of patients had received a (hemi)colectomy so that only the distal colon and rectum remained. It has been suggested that proximal HPs/SSAs have more *BRAF* mutations than distal HPs/SSAs and are more likely to develop CRC and thus more clinically relevant than distal HPs/SSAs.\textsuperscript{17, 20} Other studies however, have shown that distal HPs/SSAs have a similar frequency of *BRAF* mutations as proximal HPs/SSAs.\textsuperscript{19, 22} In addition, in the largest case series describing HPS patients, 6/10 of patients had a left-sided CRC, indicating that distal HPs/SSAs are also of clinical significance in HPS.\textsuperscript{10}
Including our 3 cases, 4 patients with MAP also meeting the criteria for HPS have now been described.\textsuperscript{10} In these patients, coincident multiple adenomas (median number 37, range: 25-40) were also present. However, bi-allelic MYH gene mutation with HPs in the absence of adenomas has never been reported. Alternatively, reported analysis on the germline MYH gene in HPS patients (n=38) revealed a bi-allelic MYH mutation in only one (3%) patient with 40 adenomas compared to a median adenoma count of only 2 (range 0-22) in the other HPS patients.\textsuperscript{10} These collective data suggest that in HPS patients MYH mutation analysis should only be considered when multiple (for example: \( \geq 25 \)) adenomas are also present. Additional studies are however required to further understand to what extent MYH testing should be performed in patients with HPS.

The adenomas in our series of MAP patients contained APC-MCR and K-ras codon 12 mutations. In HPs/SSAs of MAP patients, no
APC mutations were detected. However, in these patients we found significantly higher rates of K-ras mutations in HPs (70%) and SSAs (60%) as compared to those found in sporadic HPs (21%) and SSAs (12%) of the control group as well as to reported K-ras mutation frequencies in sporadic HPs (4-47%) \cite{19, 23-31} and SSAs (0-8%). \cite{20, 22, 23, 26}

MYH deficiency characteristically results in somatic G:C→T:A transversions in the APC and K-ras genes of adenomas.\textsuperscript{4-6} Indeed, all APC-MCR mutations detected in adenomas of our MAP patients were G:C→T:A transversions, compared to none in the control group. Accordingly, in K-ras codon 12 mutated HPs/SSAs of these patients with MYH deficiency, significantly more G:C→T:A transversions (94%) were found compared to only 29% in control group polyps. This latter percentage is concordant with previous reports of K-ras mutations in HPs from patients without a polyposis syndrome showing G:C→T:A transversions in only 0-33% of polyps.\textsuperscript{24, 30, 32} Similarly, in previous large case series of pancreatic carcinomas, having no known association with MYH deficiency, G:C→T:A transversions were observed in 6-29% of K-ras mutated carcinomas.\textsuperscript{33-35} This frequency range seems to reflect a random statistical chance, considering that there are 9 different single mutations possible in codon 12 of K-ras leading to amino acid replacement, of which 2 (22%) result in a G:C→T:A transversion (i.e. GGT→TGT or GTT).

In conclusion, our findings suggest that HPs and SSAs are causally related to bi-allelic MYH mutations. Distinct colonic polyposis pathways thus seem to prevail in at least a proportion of patients with MYH deficiency, i.e. a pathway leading to conventional adenomas with APC and/or K-ras mutations and a separate, non APC-route leading to HPs/SSAs with K-ras mutations.
REFERENCES


